VOLUME 61 *February 1950* NUMBER 2

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

CONTENTS

Streptomycin-Promizole Therapy of Miliary and Meningeal Tuberculosis in Children. EDITH M. LINCOLN and THOMAS W. KIRMSE 159

The Effect of Streptomycin upon the Pathology of Tuberculous Meningitis. WILLIAM J. WINTER 171

Tuberculous Bronchitis in Pulmonary Resection. MAURICE G. BUCKLES and WILFORD B. NEPTUNE 185

The Use of Gelatin Foam (Gelfoam) in Thoracoplasties. GUSTAF A. HEDBERG, WILLIAM D. SEYBOLD, and O. THERON CLAGETT 193

Studies on the Respiratory Gas Exchange in One Hundred Anthracite Coal Miners with Pulmonary Complaints. HURLEY L. MOTLEY, LEONARD P. LANG, and BURGESS GORDON 201

Para-aminosalicylic Acid Treatment of Tuberculosis. A Review. EMIL BOGEN 226

Case Reports:

Thrombosis of Cerebral Vessels with Necrosis of the Basal Nuclei. R. H. RIGDON and EDWARD J. LEFEBER 247

A Case of Generalized Hemangio-Sarcomatosis Erroneously Considered as Generalized Tuberculosis. MARTHA D. COLLINS and HYMAN FISHER 257

Editorial: Specific Therapy for Tuberculous Meningitis. PAUL A. BUNN 263

Letters to the Editors:

Sensitivity to Histoplasmin. RUBY G. KELLY and C. EUGENE WOODRUFF 269

Enzymatic Characteristics of Suspensions of Different Mycobacteria. HUBERT BLOCH 270

Books 272

American Trudeau Society: Report of the Laboratory Subcommittee of the Committee on Medical Research and Therapy, and of the Subcommittee on Evaluation of Laboratory Procedures of the Committee on Revision of Diagnostic Standards 274

Abstracts 29

ANNUAL MEETING—WASHINGTON, D. C.
April 25–28, 1950

See page 6

Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N. Y.

Copyright 1950, by the National Tuberculosis Association

PRINTED IN U. S. A.

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY

EDITOR-IN-CHIEF
ESMOND R. LONG
Philadelphia, Pa.

MANAGING EDITOR
WALSH McDERMOTT
New York, N. Y.

EDITORIAL BOARD

W. EDWARD CHAMBERLAIN, Philadelphia, Pa.
HALBERT L. DUNN, Washington, D.C.
H. CORWIN HINSHAW, San Francisco, Calif.
KIRBY S. HOWLETT, JR., Shelton, Conn.
JOHN C. JONES, Los Angeles, Calif.
ALEXANDER D. LANGMUIR, Baltimore, Md.
HERBERT C. MAIER, New York, N. Y.
WILLIAM P. SHEPARD, San Francisco, Calif.
C. EUGENE WOODRUFF, Northville, Mich.

HELEN W. MILLER
Editorial Secretary

NOTICE TO SUBSCRIBERS AND CONTRIBUTORS

THE AMERICAN REVIEW OF TUBERCULOSIS is published by the National Tuberculosis Association and issued monthly about the first of the month. THE AMERICAN REVIEW OF TUBERCULOSIS is the official journal of the American Trudeau Society. A volume includes six numbers and begins with the January and July numbers.

Subscription: The subscription price of the REVIEW is $10 for the calendar year. Checks should be made payable to Collier Platt, Treasurer.

Character of the Review: The REVIEW invites the submission of original manuscripts on any phase of tuberculosis, nontuberculous pulmonary diseases, and related subjects of interest.

MANUSCRIPTS

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

Manuscripts should be sent in duplicate to the office of the Managing Editor, Walsh McDermott, 1790 Broadway, New York 19, N. Y., mailed flat, and transmitted by first-class mail with postage for return if not acceptable. They should be in English, double-spaced, typewritten on one side of the page, with wide margins. Tabular data should not be included in the text but should be presented in separate tables with appropriate reference in the text. All case reports should be written in complete sentences. Authors should exercise particular care in the preparation, notation, and description of figures and tables. References should be in REVIEW style. Each table must be accompanied by a descriptive title, and a suitable legend must be provided for each figure. Drawings must be made with india ink and all lettering should be made with a stencil. The quality of the reproduction of a roentgenogram depends upon both the quality of the original picture and of the glossy print obtained from photographing that picture. If a contributor does not have facilities available, preparation of figures or reproduction of roentgenograms may be handled through the Review office. Under those circumstances, the contributor should supply a neat, properly proportioned, and suitably entitled drawing or the original roentgenogram, and will be expected to defray the cost of these services.

The publishers will not be responsible for manuscripts, illustrations, etc., lost in transit. In order to save expense for authors' corrections in proof, manuscript should be carefully revised by the author before submission.

Abstracts: Authors wishing to have abstracts of their papers appear in ABSTRACTS OF TUBERCULOSIS will facilitate their publication by sending concise abstracts or reprints to the Managing Editor.

Reprints: Fifty reprints without covers of articles will be furnished to authors free of charge when requested in advance. A table showing cost of additional reprints with an order blank is submitted with proofs to the authors.

Single Copies: The price of single copies of this number of THE AMERICAN REVIEW OF TUBERCULOSIS is one dollar postpaid.

Advertising: Rates will be furnished by THE AMERICAN REVIEW OF TUBERCULOSIS, 1790 Broadway, New York 19, N. Y. on request.

The Publishers reserve the right to decline any advertising submitted and to censor all copy. Acceptance of an advertisement does not constitute official endorsement of the product advertised.

Entered as second-class matter March 9, 1917, at the post office at Baltimore, Md., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in section 1103, act of October 3, 1917. Authorized on July 2, 1918.

sleep . . . ***measured by the patient's need***

Palatable Elixir Alurate, with its flexible, easily adjusted dosage, enables you to induce sleep in nervous, tense patients according to their individual needs. In therapeutic doses, it does not alter heart rate, respiration or other vital functions. In most cases, sleep of normal duration and depth is obtained. Easily administered, pleasant-tasting, freely miscible with liquids, each teaspoonful of Elixir Alurate contains ½ gr of allyl-isopropyl barbituric acid. Bottles of 6 oz. 1 pt. and 1 gal.

HOFFMANN-LA ROCHE INC. • NUTLEY 10 • N. J.

Elixir Alurate®

'Roche'

Zavod ANEROID PNEUMO Apparatus

THIS is a simplified apparatus for pneumothorax or other procedures requiring the introduction of measured amounts of air into the body cavities under manometric control. It consists essentially of a glass cylinder with an accurately fitted piston. By means of a hand operated cautery bulb, constant pressure is exerted on one face of the piston causing it to move and deliver air ahead of it. The movement of the piston and delivery of air can be stopped instantly by manipulation of the valve. Less than 5 cc of air can be accurately administered, as well as any desired larger amount. Therefore, the capacity of the apparatus is unlimited as it is not dependent upon the volume of a flask.

One complete excursion of the piston delivers 150 cc of air. Turning the valve immediately starts the piston in the other direction, continuing to supply air without any change of connections. Air can be delivered either slowly or rapidly as desired. An aneroid manometer is in the circuit at all times, indicating air pressure during instillation as well as the pressure in the chest when the valve is turned to zero.

Descriptive Literature and Reprints are Available

AMERICAN CYSTOSCOPE MAKERS, INC.

FREDERICK J. WALLACE, PRESIDENT

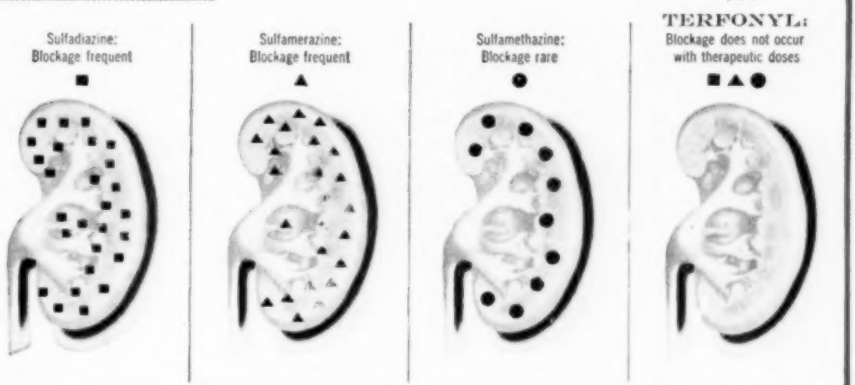
1241 LAFAYETTE AVENUE NEW YORK 59, N.Y.

TERFONYL

Sulfadiazine
Sulfamerazine
Sulfamethazine

FOR SAFE SULFONAMIDE THERAPY

Low Renal Toxicity



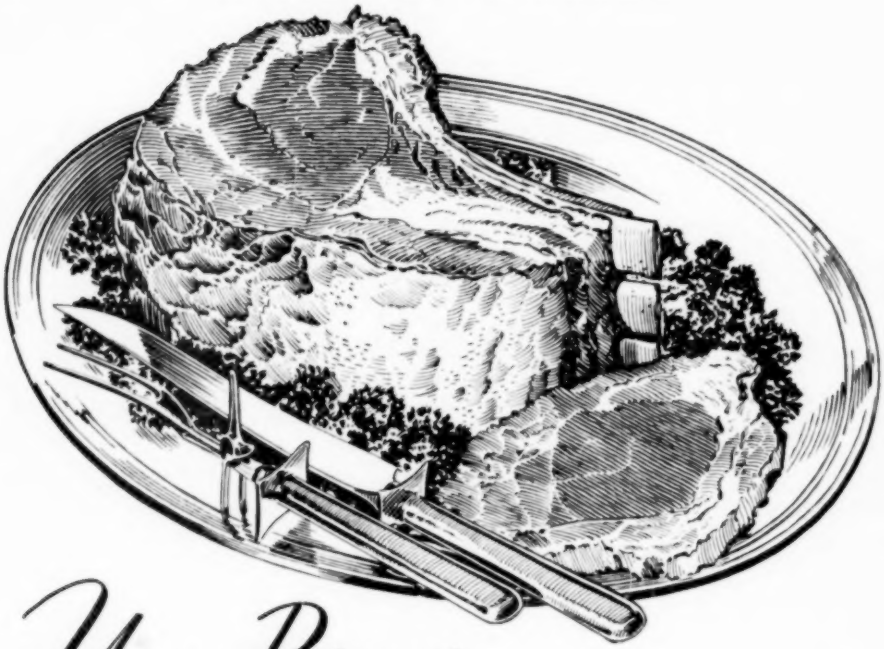
With usual doses of Terfonyl the danger of kidney blockage is virtually eliminated. Each of the three components is dissolved in body fluids and excreted by the kidneys *as though it were present alone.* The solubility of Terfonyl is an important safety factor.

Terfonyl contains equal parts of sulfadiazine, sulfamerazine and sulfamethazine, chosen for their high effectiveness and low toxicity.

Terfonyl Tablets, 0.5 Gm. Bottles of 100 and 1000

Terfonyl Suspension, 0.5 Gm. per 5 cc.
Appetizing raspberry flavor • Pint and gallon bottles

SQUIBB MANUFACTURING CHEMISTS TO THE MEDICAL PROFESSION SINCE 1858



Your Patients...

And the Meat They Eat

The established relationship between sound dietary planning and a state of maintained good health emphasizes the nutritional importance of meat, man's favorite protein food.

Not only does meat taste good, but of greater significance, it provides a host of nutritional benefits. Developments in the field of nutrition* have proved that complete protein—the kind that meat supplies in abundance—aids in building and maintaining immunity, hastens recovery after acute infectious diseases and following injury and burns, promotes health during pregnancy, aids in the growth and development of husky children, and is needed to maintain everyone in top physical condition.

No matter from what walk of life your patients come, and whether their pocketbooks demand economy or permit satisfaction of that urge for the fanciest cuts, meat gives them full value for their money.

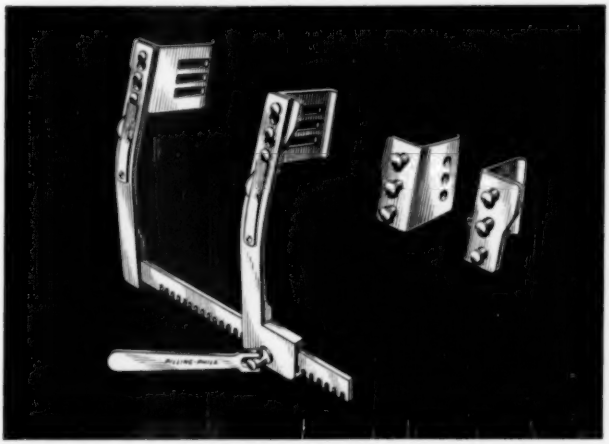
The Seal of Acceptance denotes that the nutritional statements made in this advertisement are acceptable to the Council on Foods and Nutrition of the American Medical Association.

*McLester, J. S.: Protein Comes Into Its Own, J.A.M.A. *139*:897 (April 2) 1949.

American Meat Institute

Main Office, Chicago...Members Throughout the United States

Authentic Instruments
FOR THE THORACIC SURGEON



BURFORD MODIFICATION OF THE
FINOCHIETTO RIB SPREADER

Thoracic surgeons look to Pilling for the new and important instruments in their special field. Since 1814, leaders in this field have consulted Pilling and designed instruments to fit their exacting requirements, confident in their knowledge that Pilling craftsmen would create the instruments they desired.

A fine example of Pilling craftsmanship is the Finochietto Rib Spreader, originally designed by Professors Henrique and Ricardo Finochietto of Buenos Aires. Now, the modification of this popular instrument by Thomas H. Burford, M.D., Barnes Hospital, St. Louis, provides even greater versatility. By properly switching the two pairs of interchangeable blades, the instrument can be used as medium or large size, or combinations of one large and one small blade. The shafts are also curved to conform to body curvature, thus keeping the spreader out of the operator's way.

ORDER instruments direct from

George P. Pilling and Son Company

3451 WALNUT STREET
Philadelphia

A Standing Invitation: When in Philadelphia, visit our modern sales rooms and manufacturing plant. Free parking on our private lot.

INSTRUMENTS BY Pilling

PILLING FOR PERFECTION in surgical instruments

SAROT
Bronchial Clamp
Available in pairs—
right and left

SAROT
Intra-Thoracic
Artery Clamp

SATINSKY
Scissors

SATINSKY
Vena Cava Clamp

EUDOWOOD SANATORIUM

Towson, Maryland

A modern, thoroughly equipped institution for the treatment of tuberculosis. Located on a large 350-acre farm in the beautiful, healthful Maryland countryside. All city conveniences . . . eight miles north of Baltimore . . . one mile east of Towson, Maryland.

Private rooms with adjoining baths . . . $7.00 and $10.00 per day, including general nursing and medical attention. ***For further information, address:***

DR. WILLIAM A. BRIDGES, ***Superintendent***
Eudowood, Towson 4, Maryland

1950 National Tuberculosis Association Annual Meeting
Washington, D. C.

The Annual Meeting of the National Tuberculosis Association will be held in Washington, D. C. April 24–28, 1950. Meeting sessions will be held in the Hotel Statler.

Some 920 rooms have been promised by the hotels listed below for the accommodation of those attending the meeting. Only a limited number of single rooms will be available.

Application for room reservations should be made directly with the hotel of your choice and should be made for double rooms wherever possible.

HOTEL	*ADDRESS*	*SINGLE*	*DOUBLE*
STATLER	16th & K Streets, N.W.	$5.00–$11.50	$8.00–$12.00
AMBASSADOR	14th & K Streets, N.W.	3.50– 6.00	6.00– 8.00
BLACKSTONE	1016 17th Street, N.W.	4.00– 5.00	6.00– 7.00
HAMILTON	14th & K Streets, N.W.	4.00– 5.50	6.00– 7.00
HAY-ADAMS	16th & H Streets, N.W.	4.50– 7.00	6.50– 8.00
LAFAYETTE	16th & Eye Streets, N.W.	5.00– 7.00	7.00– 10.00
MARTINIQUE	16th & M Streets, N.W.	4.00– 6.00	6.00– 8.00
SHERATON	15th & L Streets, N.W.	3.85– 5.85	6.35– 8.85
WASHINGTON	15th & Pa., N.W.	5.00– 9.00	9.00– 12.00
WILLARD	14th & Pa., N.W.	4.50– 8.00	7.00– 11.00

See February issue of N.T.A. Bulletin for preliminary programs

SCHENLEY LABORATORIES

announces the availability of

TIBIONE

brand of 4-acetylaminobenzal thiosemicarbazone

a new antimicrobial agent for tuberculosis

developed by DRS. DOMAGK, SCHMIDT, BEHNISCH, and MIETZSCH of the BAYER LABORATORIES at Leverkusen and Elberfeld, Western Zone of Germany

Known abroad as TB1/698, TIBIONE has been used in treating more than 7,000 tuberculosis patients in Europe and is now being extensively studied by leading tuberculosis groups in the United States, using material supplied by Schenley Laboratories, sole American licensee. Clinicians and institutions desiring to purchase TIBIONE for use in clinical investigation under F & D A regulations are invited to communicate directly with the Medical Director of Schenley Laboratories.

SCHENLEY LABORATORIES, INC.

350 Fifth Avenue, New York 1, N. Y.

© Schenley Laboratories, Inc.

	Calories	Protein Gm.	Calcium Gm.	Copper mg.	Iron mg.	Phosphorus Gm.	Vitamin A I. U.	Thiamine mg.	Riboflavin mg.	Niacin mg.	Ascorbic Acid mg.	Vitamin D I. U.
National Research Council Allowances. Sedentary Man (154 lbs.)	2,400	70	1.0	1.2	12	1.5	5,000	1.2	1.8	12	75	Small Amount
Ovaltine in Milk, 3 Servings *	676	32	1.12	0.5	12	0.94	3,000	1.16	2.0	6.8	30	417
Percentages of N. R. C. Allowances Provided by 3 Servings* of Ovaltine in Milk	28%	46%	112%	42%	100%	63%	60%	97%	111%	57%	40%	Abundance

*Each serving made of ½ oz. of Ovaltine and 8 fl. oz. of whole milk.

In Protecting Dietary Adequacy

of the tuberculous patient

The *multiple nutrient dietary food supplement*, Ovaltine in milk, is highly qualified for protecting the nutrient adequacy of the diet of the tuberculous patient. This is strikingly obvious from the data in the above table listing:

(1) The National Research Council's Recommended Daily Dietary Allowances (revised 1948) for a sedentary man of 154 lbs.,

(2) The nutrient content of three servings of Ovaltine in milk, and

(3) The percentages of the Council's Dietary Allowances provided by the three servings of Ovaltine in milk.

Enticing flavor and easy digestibility are other features of Ovaltine in milk important to the tuberculous patient. During periods of food resistance, which may constitute a severe threat to the nutritional state, this delicious dietary supplement is particularly valuable.

Two kinds, Plain and Sweet Chocolate Flavored. Serving for serving, they are virtually identical.

Ovaltine

THE WANDER COMPANY, 360 N. MICHIGAN AVE., CHICAGO 1, ILL.

• 1949 Issues Needed •

We will pay $1.00 per copy for any of the following issues, if they are in good condition. Mail them to the address below for prompt payment.

January, 1949
February, 1949
March, 1949

AMERICAN REVIEW OF TUBERCULOSIS

1790 Broadway, New York 19, New York

Industry, Tuberculosis Silicosis & Compensation

160 pages, clothbound, $2.00 postpaid

Edited by **Leroy U. Gardner, M.D.,** this useful volume assembles thirteen papers outlining significant developments in the handling of industrial X-ray surveys and follow-ups; in the identification and treatment of silicosis; in the field of workmen's compensation.

Recommended for thoughtful reading by industrial physicians, tuberculosis specialists, business and insurance executives.

Send your order today to the

National Tuberculosis Association
1790 Broadway New York 19, N. Y.

Present Concepts of Rehabilitation in Tuberculosis

by **NORMAN C. KIEFER, M.D.**

420 pages, cloth cover $3.50

In this volume there is brought together, for the first time, an accurate summary of world research and achievement in every area of rehabilitation during the last decade.

More than 1,000 original papers are abstracted in this book, and in every case the salient features of each have been brought together in classified sections to provide a complete picture of approaches, procedures, and results.

A bibliography of more than 1,000 items supplies reference to basic sources, and a particularly comprehensive index facilitates the location of specific information.

Your order can be filled promptly.

NATIONAL TUBERCULOSIS ASSOCIATION
1790 Broadway, New York 19, N. Y.

Reduce Costs . . . Improve Service with

INTERFOLDED SPUTUM NAPKINS AND DISPENSERS

Where records have been kept, it has been established that the use of Interfolded Sputum Napkins and Dispensers saves money for sanatoria. That is point No. 1 in their favor.

Patients like the neat, handy dispenser service. That is point No. 2 in their favor.

Made of excellent stock, especially selected and especially creped for Sputum Napkins, they are far more sanitary than cloth, gauze or cellulose. That is point No. 3 in their favor.

Napkins are 6½ x 6½ inches, interfolded, and packed 250 to a package. Made of excellent stock, especially for this purpose, creped especially soft. Handsome, neat dispensing cabinet finished in light green Duco, dispenses two napkins at a time. Cabinet has rubber feet to prevent marring furniture.

If you are not already using Interfolded Sputum Napkins and Dispensers, ask your Will Ross., Inc., representative for complete details.

WILL ROSS, INC.

Tuberculosis Sanatorium Supplies and Equipment

Milwaukee 10, Wisconsin

ERRATA

In Volume 60, No. 3, 1949, "An Appraisal of the Contribution of Mass Radiography in the Discovery of Pulmonary Tuberculosis", Table 5, the heading of the last column should read "Number Probably Inactive."

In Volume 60, No. 5, 1949, "Variation in the Duration of Tuberculin Skin Sensitivity Produced by Two Strains of BCG," in the third paragraph on page 545 the third sentence should read "No individual was said to have sensitivity to tuberculin unless an area of induration measuring at least 5 by 5 mm. was present at the end of forty-eight hours."

In Volume 60, No. 3, 1949, "Tuberculosis in Nurses: Clinical Observations on its Pathogenesis as Seen in a Fifteen Year Follow-up of 745 Nurses," a misquotation of the *Prophit Tuberculosis Survey Report* has been called to our attention and acknowledged by the authors as follows:

Marc Daniels—Medical Research Council
Tavistock House North
Tavistock Square
London, W. C. 1

16th November, 1949

Dr. Walsh McDermott
Managing Editor
American Review of Tuberculosis
1790 Broadway

Dear Dr. McDermott,

May I call your attention to a serious misquotation of the Prophit Tuberculosis Survey Report, in an article by Drs. Badger and Ayvazian in the September 1949 number of the American Review of Tuberculosis (page 321). The authors quote as follows: Prophit Tuberculosis Survey, page 184: "It is recognised by all, except a few who believe that pulmonary tuberculosis in young adults is usually the result of a primary infection, that reinfection is responsible for much or indeed most adult tuberculosis."

In the original text "recent" appears before the words "primary infection." The misquotation is serious, because it is obvious that there are not a few, but a great many people (of the "endogenous reinfection or exacerbation" school of thought) who believe that all tuberculous disease develops from the bacilli of primary infection, whether the date of that infection is recent or remote. Our reference was to a few workers who believe that most tuberculosis in young adults arises from a *recent* primary infection; this may apply to the few countries where infection rates are now low as in some states of the U.S.A., but it certainly does not apply to this country, nor to most other European countries.

With best regards,

Yours sincerely,
Marc Daniels

Dr. Walsh McDermott
Managing Editor
American Review of Tuberculosis
1790 Broadway
New York 19, N. Y.

Dear Dr. McDermott:

We are glad to have the correction of the misquote that was wholly accidental, and we regret its occurrence. The comment by Marc Daniels does, perhaps, point out still more clearly the differences in our thinking on the question of the pathogenesis of tuberculosis. But however we and our British confreres may differ on these academic points, we remain staunch friends on the matter of treatment and prevention of tuberculosis. Space does not permit adequate discussion of this extremely interesting core of the problem. We reiterate our emphasis of the inherent constitutional factor of resistance or susceptibility to tuberculosis as being the most important influence in establishing the pattern of disease. This is based on our long clinical observations plus the very significant and brilliant experimental studies of this subject by Lurie.

Sincerely,
Theodore L. Badger, M.D.

THE AMERICAN REVIEW
OF
TUBERCULOSIS

VOLUME 61 *February 1950* NUMBER 2

STREPTOMYCIN-PROMIZOLE THERAPY OF MILIARY AND MENINGEAL TUBERCULOSIS IN CHILDREN[1,2]

EDITH M. LINCOLN AND THOMAS W. KIRMSE

(Received for publication August 11, 1949)

INTRODUCTION

Streptomycin, the most effective tuberculostatic agent available, has had extensive clinical trial. Prior to its use the fatality rate in miliary and meningeal tuberculosis was very high. Preliminary reports of streptomycin therapy in these forms of tuberculosis, based on results soon after cessation of treatment, were favorable. After more prolonged observation, however, the end results were not as good as had been anticipated.

Additional experimental and clinical information has rapidly accumulated concerning the limitations of streptomycin when employed as a sole therapeutic agent. The main disadvantages of streptomycin are the selective toxicity for the eighth nerve, especially the vestibular branch, and the tendency for drug-resistant tubercle bacilli to emerge to predominance after prolonged use of the antibiotic. In other forms of tuberculosis, reduction of the daily dose of streptomycin and short-term administration have been reported without loss of therapeutic effect and with less serious toxic reactions. In miliary and meningeal tuberculosis, however, long-term administration of the drug is mandatory; toxic effects and the development of bacterial resistance may be expected to continue.

Before streptomycin had been discovered, sulfones such as Diasone, Promin, and Promizole were found to have a suppressive effect in experimentally induced tuberculosis in guinea pigs. Feldman, Hinshaw, and Mann (1) treated tuberculous guinea pigs with Promizole, and the disease was converted to an arrested or non-

[1] From the Chest Clinic of the Children's Medical Service, Bellevue Hospital, New York City, and the Department of Pediatrics, New York University-Bellevue Medical Center.

[2] This study was aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service, a grant from the Committee on Medical Research and Therapy of the American Trudeau Society, Medical Section of the National Tuberculosis Association, and a grant from Parke, Davis & Company.

progressive form with resolution of the morbid processes. Feldman, Hinshaw, and Pfuetze (2), after completing a series of experiments with sulfones, concluded that Promizole is at least as effective as any previously described compound but is unique in being easily administered, well absorbed, and of extremely low toxicity for human beings.

In the chest clinic of the Children's Medical Service, Bellevue Hospital, the use of Promizole was started in 1944 (3, 4, 5). Forms of tuberculosis with very grave prognosis were first selected for treatment. Five cases of tuberculous meningitis were treated; all died without apparent effect on the course or duration of the disease. Treatment of acute hematogenous miliary tuberculosis was then attempted. Originally, there were 11 patients in this group. One was dropped from the group because after further study it was decided that she did not have an acute generalized hematogenous tuberculosis. Of the 10 remaining patients, 3 were treated only twelve to twenty-six days and were therefore not included in the results. Of the 7 patients treated more than one month, 5 showed roentgenographic evidence of complete clearing of the miliary tuberculosis within six to twelve weeks and have remained well three to five years. The other two children died forty-three days and four and one-half months, respectively, after treatment was initiated.

The duration of treatment of these cases of miliary tuberculosis with Promizole was arbitrarily set at three years. The slow rate of disappearance of the miliary tubercles on the roentgenogram showed the need for prolonged therapy. Moreover, Feldman, Hinshaw, and Moses (6) had shown that the spleens of animals apparently cured by Promin yielded viable tubercle bacilli. The need for prolonged bacteriostatic action was also suggested by the reports of Medlar and Sasano (7) that relapses in animals were frequent after withdrawal of Promin.

After the discovery of streptomycin, numerous experiments were recorded in which this antibiotic was used in combination with a sulfone. Smith (8) observed the influence of streptomycin and Promin on proliferation of tubercle bacilli in the tissues of the albino rat and concluded that these agents administered together exerted a chemotherapeutic action greater than what might have been anticipated from simple summation of effects. Callomon (9) combined streptomycin and Diasone and obtained better results in the treatment of tuberculosis in guinea pigs than with either compound alone, suggesting the operation of synergistic or additive therapeutic effects. This experimental evidence of the value of combining a sulfone with streptomycin was one reason why, in December 1946 when streptomycin became available to us, it was decided to combine it with Promizole in the treatment of miliary and meningeal tuberculosis. It was also hoped that the use of two different types of therapeutic agent might delay or diminish the emergence of drug-resistant tubercle bacilli. Promizole was chosen as the drug to supplement streptomycin because definite clinical evidence of its inhibitory effect had already been established. By combining streptomycin and Promizole the rapid action of streptomycin could be utilized; bacteriostasis could then be continued for a long time by Promizole as the sole chemotherapeutic agent.

RESULTS

Treatment of Miliary Tuberculosis with Streptomycin and Promizole

Thirteen children with miliary tuberculosis were treated with both drugs (table 1).[3] Two are still under treatment. Seven children have been observed from 10 to 27 months after starting treatment and remain well. All but one of the cases surviving received streptomycin intramuscularly 1.0 Gm., daily for

TABLE 1

Results of Treatment of Miliary Tuberculosis with Streptomycin and Promizole

CASE NUMBER	AGE	COMPLETE REGRESSION MILIARY LESIONS	SURVIVAL* TIME	COMMENT
1	11 years	7 weeks	27 months	Developed meningitis (Case 1; table 3).
2	4 years	7 weeks	11 months	Developed meningitis (Case 3; table 3).
3	5 years	10 weeks	19 months	Thrombopenic purpura on admission; full clinical recovery.
4	9 months	8 weeks	18 months	Endobronchial tuberculosis with RML collapse; full clinical recovery.
5	8 months	18 weeks	18 months	Meningitis on admission (Case 9; table 3); measles, slowly clearing miliary.
6	8 months	8 weeks	15 months	Full clinical recovery.
7	6 months	—	—	Pertussis 2 months prior to admission; died sixth treatment day.
8	13 months	12 weeks	14 months	Measles 6 weeks prior to admission; developed meningitis (Case 11; table 3).
9	20 months	8 weeks	10 months	Full clinical recovery.
Patients treated since January 1949. Dihydrostreptomycin and Promizole used				
10	11 months	12 weeks	5 months	Endobronchial tuberculosis with collapse LLL; good clinical condition.
11	11 months	11 weeks	4 months	Good clinical condition.
12	9 months	7 weeks	3 months	Meningitis on admission (Case 21; table 3); still under treatment.
13	3 months	—	2 months	Still under treatment.

* Computed from beginning of treatment.

120 days; case 4 received only 0.4 Gm., daily. Promizole is given orally four times daily in amounts sufficient to obtain concentrations in the blood of 1.0 to 3.0 mg. per 100 cc. An initial dose of 1.0 Gm., daily is given, and in the absence of signs of toxicity the drug is gradually increased until the desired blood concentration (taken three hours after the last dose of Promizole) is obtained. Since the rate of absorption and excretion of Promizole varies with the individual patient, dosage and therapeutic levels cannot be determined on a weight basis. It has been noted repeatedly that children in the two- to five-year-old group frequently

[3] Cases 1 to 9 have been previously reported (5). One case (listed as No. 16 in this previous publication) has now been omitted from the series because on further study the diagnosis of acute generalized miliary tuberculosis could not be clearly established.

attain high concentrations of Promizole in the blood on small daily doses (0.375 to 2.0 Gm.), while infants and older children may require up to 8.0 Gm. daily to attain adequate levels. It is planned to continue treatment with Promizole for three years.

As determined by roentgenographic examination, the rate of clearing of the miliary tubercles was much more rapid with combined therapy than with Promizole alone. The average interval between the start of therapy and disappearance of miliary mottling was eight months with Promizole alone and nine weeks with combined therapy. None of the children treated with Promizole alone developed clinical meningitis; 2 patients developed it after six and twelve weeks, respectively, of combined therapy. No relapse of miliary tuberculosis occurred in either group.

TABLE 2

Plan of Treatment—Miliary and Meningeal Tuberculosis

I. Miliary Tuberculosis:		
Streptomycin	—intramuscularly	—1.0 Gm., daily for 120 days.
Promizole	—orally	—1.0 to 8.0 Gm., daily for 3 years.
II. Tuberculous Meningitis:		
Streptomycin	—intramuscularly	—1.0 Gm., daily for 180 days.
	—intrathecally	—0.1 to 0.05 Gm., for series of 40 injections.
Promizole	—orally	—1.0 to 8.0 Gm., daily for 3 years.
III. Combined Miliary Tuberculosis and Tuberculous Meningitis:		
When present simultaneously, treated as meningitis.		
When meningitis develops in course of treatment for miliary tuberculosis, the full course of treatment for tuberculous meningitis is begun.		
IV. Relapse:		
Repeat original course of therapy.		

Tuberculous Meningitis

From December 1946 through July 1949, 21 consecutive patients with tuberculous meningitis were treated according to the plan shown in table 2. Cases 1 to 7 were first reported in 1948 (10) and cases 1 to 18 were cited in 1949 (5). Three additional patients still receiving streptomycin are included in the series presented at this time. The status of the 21 patients as of July 1, 1949 is given in table 3. Sixteen children survive; there were 5 deaths, including the child with miliary tuberculosis who later died of meningitis (Case 3).

The age of the patients ranged from eight months to eleven years at the time treatment was started; 14 patients were three years old or less. The source of infection was determined in 12; only 7 children had been under observation previously for tuberculosis. Reaction to the Mantoux test was positive in all patients. Roentgenographic evidence of manifest primary tuberculosis was present in 10 children; 6 others showed a calcified primary complex and the remaining 5 had acute hematogenous tuberculosis. The first symptoms of meningitis developed within six months of the onset of primary infection in 11 patients. Three children had measles, another probably had whooping cough within a

TABLE 3

Results of Treatment of Tuberculous Meningitis with Streptomycin and Promizole

CASE NUMBER	AGE	ROENTGENOGRAPHIC EVIDENCE OF TUBERCULOSIS	STAGE MENINGITIS WHEN DIAGNOSED	SURVIVAL* TIME	COMMENT
1	9 months	Primary	Late	—	Hydrocephalus on admission; died sixty-sixth day of treatment.
2	11 years	Miliary and Spondylitis	Early	27 months	Appendectomy during tenth month; two-stage spinal fusion sixteenth and eighteenth months; good clinical condition.
3	4 years	Miliary	Early	—	Relapsed 24 days after treatment completed. Died during fourth month of relapse.
4	3 years	Primary	Early	26 months	Doing well at home.
5	2 years	Primary	Early	25 months	Deaf, blind with full restoration; doing well at home.
6	6 years	Primary	Late	24 months	Severe encephalitis with slow improvement; impairment of hearing; now well at home.
7	23 months	Primary	Early	22 months	Doing well at home.
8	3 years	Calcified primary	Moderately advanced	20 months	Relapsed twice: 43 and 44 days after completing treatment; deaf; residual weakness right hand and leg; general good condition.
9	8 months	Miliary	Early	18 months	Residual weakness right arm and leg; good clinical condition; in convalescent home.
10	5 years	Calcified primary	Early	18 months	Relapsed 45 days after completing treatment; left foot drop; doing well at home.
11	13 months	Miliary	Early	13 months	Deaf; doing well.
12	6 years	Calcified primary	Moderately advanced	13 months	Hearing fully recovered; doing well at home.
13	10 months	Primary	Late	—	Died tenth day of treatment.
14	11 months	Primary	Late	—	Died seventeenth day of treatment.
15	15 months	Primary	Moderately advanced	12 months	Weakness left leg; doing well at home.
16	5 years	Calcified primary	Early	11 months	Doing well at home.

TABLE 3—*Concluded*

CASE NUMBER	AGE	ROENTGENOGRAPHIC EVIDENCE OF TUBERCULOSIS	STAGE MENINGITIS WHEN DIAGNOSED	SURVIVAL* TIME	COMMENT
17	13 months	Primary	Moderately advanced	—	Died thirty-ninth day of treatment; exfoliative dermatitis.
18	2 years	Calcified primary	Moderately advanced	9 months	Transient hemiplegia during first month; general good condition.
19	4 years	Calcified primary	Late	4 months	Transient hemiplegia during first month; still under treatment.
20	16 months	Primary	Early	3 months	Still under treatment; doing well.
21	9 months	Miliary	Moderately advanced	3 months	Deaf and blind on admission—both improving; still under treatment.

* Computed from beginning of treatment.

two-month period prior to admission. Tonsillectomy and head injury may have been precipitating factors in the development of meningitis in 3 other children. The first signs of meningitis developed under ward observation in 9 patients; 3 of these were already being treated for miliary tuberculosis. Otitis media and acute respiratory infection are present frequently in the early course of illness (9 patients in this series) and may obscure the real cause of fever and other early symptoms. Three patients developed strabismus, 3 had convulsions prior to treatment; all patients who died had terminal convulsions.

All patients were treated with streptomycin, intramuscularly and intrathecally, and with Promizole by mouth. Though the general plan of treatment was the same in all cases, individual variations were inevitable. The clinical experience gained from our first cases has helped to establish our present plan of treatment. Unfavorable reactions to streptomycin and Promizole have been infrequent. Streptomycin intramuscularly is usually given twice daily in 0.5 Gm. doses at 8 a.m. and 4 p.m. A single daily dose can be tolerated by large or well-nourished children. Streptomycin intrathecally is given once daily in 0.1 Gm. doses until toxic symptoms or mechanical difficulties arise. Thereafter, intrathecal treatment is administered every two days. If toxic symptoms again arise, the dosage is reduced to 0.05 Gm., daily or every two days until a total of 40 intrathecal injections is completed. Promizole is administered in the same manner prescribed for miliary tuberculosis.

Even when streptomycin therapy is commenced early, the child usually passes into the second stage of tuberculous meningitis and exhibits the neurologic signs associated with the disease. At first, the course may be similar to that of the untreated patient and the child may appear not to be benefiting from treatment. Fever is slow to decline. Evidence of cranial nerve involvement clears slowly; diminished to absent superficial and deep reflexes may persist throughout the course of treatment.

Six survivors had hemiplegia with facial paralysis; in 2 of these patients the findings were transient and cleared entirely within a few weeks; the others have residual paralyses but have improved remarkably during convalescence. Some degree of increased tone in the hamstring muscles was apparent in most cases but this gradually disappeared with increased activity. All survivors have normal mentality.

Three patients relapsed 25 to 45 days after completing six months of treatment with streptomycin and Promizole. Relapse was diagnosed on the basis of cerebrospinal fluid changes before any signs or symptoms of meningitis recurred. Tubercle bacilli fully sensitive to streptomycin were cultured from the cerebrospinal fluids in spite of the fact that they had received the highest total dosages of streptomycin for patients in the series. All 3 patients with relapse were retreated. These 3 children are the only ones whose electro-encephalograms show definite focal lesions. One child (Case 10) has completed treatment for relapse and, except for a slight limp, is in good condition at home. Another patient (Case 8) relapsed twice and has just completed a third course of treatment. He is totally deaf, has a residual paralysis of the right leg and arm, but appears in excellent health. The third child who relapsed (Case 3) had miliary tuberculosis on admission. Tubercle bacilli were cultured from the blood nine days after streptomycin was started by the intramuscular route. After the miliary tuberculosis had cleared on the roentgenogram, meningitis developed and the intrathecal administration of streptomycin was started. The patient's cerebrospinal fluid became normal before the end of therapy and he appeared well and active. Twenty-five days after streptomycin was discontinued, relapse was diagnosed by cerebrospinal fluid changes in the absence of any neurologic signs. During the second month of therapy for the relapse, tubercle bacilli became fully resistant to streptomycin, rapid deterioration followed, and the child died eleven months after miliary tuberculosis was diagnosed.

Toxic Effects of Combined Streptomycin and Promizole Therapy

The toxic effects of Promizole when given alone have been discussed previously. It is possible that the acute symptoms of toxicity were exaggerated in our first cases because the dosage was rapidly increased. Either because the dosage is now increased gradually and only until a therapeutic blood level is reached, or possibly as a result of combined therapy, there seems to be little evidence of toxic reaction to Promizole in patients with miliary or meningeal tuberculosis. The only reactions now commonly encountered are cyanosis and slight enlargement of the thyroid gland. Promizole is definitely goitrogenic. On combined therapy, however, enlargement of the thyroid gland has been minimal and only sometimes associated with an elevation of the blood cholesterol concentration. Cyanosis, slight or moderate, may appear as early as a few hours after the first dose of Promizole. Some children remain cyanotic as long as medication is continued. In several children with acute febrile illnesses and low fluid intake, cyanosis has become intensified. Promizole values obtained in the blood at this time have been high. A cherry-red or pink urine is frequently seen in children taking the drug, and reducing substances may also be present. One child (Case

6) showed enlargement of nipples and breast development after eight months of treatment. On combined therapy no toxic effects requiring cessation of Promizole treatment were noted. All of the effects which were observed were reversible with the possible exception of the stimulation of secondary sex characteristics.

The most constant toxic reaction to streptomycin is ataxia. Every patient showed some degree of unsteadiness after three or four weeks of treatment. Response to caloric stimulation tests (Kobrak) was invariably lost, sometimes as early as the second week of therapy. Vestibular recuperation was slow after treatment was discontinued but of the 5 meningitis patients with the longest follow-up observations, 4 have normal vestibular reactions; the fifth has a spontaneous nystagmus. In spite of persistence of vestibular dysfunction, the disturbance of equilibrium subsided in most of the other patients, and they run and play like normal children.

The most striking toxic effects were observed after the administration of streptomycin intrathecally. In some instances, increased vertigo, ataxia, transient nystagmus, and strabismus as well as projectile vomiting developed within one to four hours. Occasionally, opisthotonos, convulsions, muscle rigidity, or sudden high temperature were seen. After these more severe reactions, the patients usually became drowsy and fell into a quiet sleep. On awakening there was full recovery from these alarming symptoms although complete loss of reflexes with muscle flaccidity has persisted for 24 hours or more.

Other toxic effects were similar to those reported elsewhere. Four children had generalized rashes, in 2 cases associated with eosinophilia; one of these developed exfoliative dermatitis and died (Case 17). Post-mortem examination revealed the presence of severe tuberculous meningitis, miliary tuberculosis, and generalized exfoliative dermatitis. One child (Case 5) had a temporary elevation of the blood nonprotein nitrogen with albuminuria and cylindruria. He also became deaf and blind during the first month of treatment but vision and hearing were completely restored without reducing dosage and before therapy was ended. Four other patients became deaf while under treatment; one of them fully recovered his hearing.

THE CEREBROSPINAL FLUID

Cerebrospinal fluid sugar values were normal in 4 patients at the time meningitis was diagnosed; 3 of these had miliary tuberculosis and 2 of them were already receiving streptomycin intramuscularly. All patients in the series had low cerebrospinal fluid concentrations of sugar (22 mg. per 100 cc. or less in 13 patients) early in the course of treatment but the values were restored to normal in most cases within a week or two, and in the others by the end of intrathecal therapy. The value for total protein remained elevated in only 5 survivors after streptomycin therapy had been completed; 2 of these relapsed. The cerebrospinal fluid cell counts showed much variation and usually did not decline to 10 or less per cu. mm. before the sixth month. Both cells and protein remained elevated in the patients who died. In virtually all cases, cultures for tubercle bacilli became negative within a few days after the intrathecal admin-

istration of streptomycin was begun, and in only 3 patients who relapsed were positive cultures obtained after termination of the antibiotic. The cerebrospinal fluid chloride levels declined slowly and showed too much variation to be of any diagnostic or prognostic significance and hence are no longer determined as a routine procedure.

Because the diagnosis of tuberculous meningitis may be obscured in patients who are already under streptomycin therapy, other tests of the cerebrospinal fluid which may help to confirm the diagnosis are being investigated. Enough evidence has accumulated to indicate that in the presence of inflamed meninges, a fairly high concentration of streptomycin (5 to 40 γ per cc. or more) appears in the cerebrospinal fluid when the drug is given intramuscularly. As the disease subsides, the concentration of streptomycin tends to decline. With continued clinical improvement, most patients who were still receiving streptomycin intramuscularly have shown cerebrospinal streptomycin levels of 1 to 0 γ per cc. during the third to fifth month of therapy. Similar streptomycin levels (1 to 0 γ per cc.) were repeatedly obtained in 16 patients treated with streptomycin intramuscularly who at no time showed any clinical or laboratory evidence of meningitis. In the presence of tuberculous meningitis, the Levinson test is positive and the colloidal gold curve abnormal. As the disease subsides in response to therapy, the Levinson test becomes negative and the colloidal gold curve returns to normal. Such aids may be particularly important in the early recognition of relapse. The trend of these three tests, cerebrospinal fluid streptomycin levels, Levinson, and colloidal gold, may ultimately help in determining the duration of therapy. All were negative as early as the fourth month in at least one patient and during the fifth and sixth months in most of the others tested. At present we are investigating the diagnostic and prognostic value of tryptophane levels in the spinal fluid.

DISCUSSION

Conclusive evidence that streptomycin therapy has reduced the very high mortality in miliary and meningeal tuberculosis is apparent from the number of survivors reported (table 3). It is of interest to note that in the few available reports in which more than one drug was used, the results are more favorable than those obtained with streptomycin alone. The possibility that combined therapy may also be more efficacious in other forms of tuberculosis is suggested by the studies of Woods and Wood (11). They concluded that the combination of streptomycin and Promizole has a more pronounced clinical and decidedly greater bactericidal action in experimental ocular tuberculosis than either agent used separately.

The value of combined therapy in meningitis can perhaps best be judged by the rate of survival and the physical and mental condition of the survivors. Since drug-resistant strains develop much less frequently in meningitis than in other forms of tuberculosis, it is unlikely that evidence from patients with meningitis will prove that combined therapy delays or diminishes the emergence of resistant organisms. In pulmonary tuberculosis such proof has already been published (12).

The most informative change in the cerebrospinal fluid constituents is a low or declining value for sugar. An increase in cells and protein content above normal and a sugar content below normal, in the absence of other organisms, provides a tentative diagnosis of tuberculous meningitis. The chlorides decline too slowly and too late to be of any diagnostic value. If these findings are confirmed in 24 to 48 hours and the presence of other bacteria has been excluded, active treatment should be initiated while further confirmation is sought by culture and guinea pig inoculation of the cerebrospinal fluid.

The clinical picture of tuberculous meningitis may be temporarily obscured if the patient is already receiving streptomycin intramuscularly as in miliary tuberculosis. All patients with miliary tuberculosis are potential cases of meningitis and periodic lumbar punctures should be done. Increase in the number of cells and a rise in the protein content are of particular value when the sugar content is normal. In 4 patients with miliary tuberculosis the cerebrospinal fluid of 3 showed normal sugar content at the time of diagnosis although tubercle bacilli were obtained by culture. In such cases where the patient is already on streptomycin therapy, elevation of the streptomycin cerebrospinal fluid level may be of great diagnostic value since levels tend to be 0 to 1 γ per cc. in the absence of meningitis.

The slow response of the child to therapy may be due in part to the irritant effect of intrathecal therapy. Nevertheless, the percentage of clinical remissions obtained by intramuscular therapy alone is not high enough to advocate treatment solely by this route. The report of the British Medical Research Council (13) clearly demonstrated that the number of cases cured by combined intramuscular and intrathecal therapy is greater than with intramuscular therapy alone. Intrathecal therapy should be intensive from onset in order to obtain high concentrations of streptomycin in the cerebrospinal fluid before the tubercle bacilli become imbedded in gelatinous exudate.

That children safely tolerate proportionately larger doses of chemotherapeutic agents than adults has been repeatedly observed in sulfonamide and penicillin therapy and, perhaps, is true likewise of streptomycin. In the present series of patients with miliary or meningeal tuberculosis treated with combined therapy, streptomycin 1.0 Gm. daily by the intramuscular route was well tolerated even in relapse. In only 6 of the 16 survivors was 0.1 Gm. intrathecally tolerated for the full series of 40 injections. In the others, because of toxic reactions, the dosage was reduced to 0.05 Gm., but it has not been necessary to make any reduction below this even in the smallest patients. Promizole is very well tolerated. We have not observed gastric irritation, nausea, or vomiting when the drug is crushed and sweetened for palatability. Promizole must be given in therapeutic amounts if the best results are to be expected. For the present, this amount must be determined by the concentrations of Promizole attained in the blood because the absorption and excretion rate varies with the individual patient. Maximum Promizole values are reached in the blood approximately three hours after the last oral dose. In almost all cases, once the required dosage is determined, the same dose must be continued to obtain comparable levels.

In our small group of patients it is impossible to evaluate the significance of

age of the child in prognosis. It is suggestive, however, that of the 5 deaths from meningitis, 4 patients were less than fourteen months old and in a moderate to far advanced stage of the disease at the time treatment was started. Of 10 children with meningitis aged two years or less, 4 died within the first ten weeks of treatment.

In order to carry out effective treatment, a cooperative program for the care of children with miliary and meningeal tuberculosis has been organized. Of foremost importance is good nursing care. Electro-encephalograms are done as a routine procedure and are repeated later in the course of the disease. Psychiatric evaluation is obtained on each patient. When paralyses of extremities develop, supervision of treatment, including physiotherapy, is done jointly by the orthopedic and rehabilitation services. Since some children develop meningitis before they have learned to walk or talk and may have the further handicap of ataxia or impaired hearing secondary to treatment, a plan for their rehabilitation is essential. Moreover, recreational and physical activities are important not only to prevent the traumatic experience of the long hospital stay but to aid in the full physical and mental evaluation of the child recovered from miliary and meningeal tuberculosis.

It is difficult to compare the results of different observers because each group shows so much variation in dosage, length of treatment, and follow-up. Added to this difficulty is the frequency with which miliary tuberculosis and tuberculous meningitis are massed together as though they were one and the same disease. Considering the difference in prognosis, it is reasonable to separate reports on the basis of original diagnosis. Only by so doing can the problem of adequate treatment be evaluated on a sound basis. The classification used by Bunn (14) is admirable for a cooperative study but would discourage the individual clinician from reporting his few cases. It becomes increasingly important to report failure as well as success in order to obtain significant data. Only by such comparison and exchange of ideas can the most effective regimen be evolved with the therapeutic agents now available. The goal of all is identical: a large percentage of eventual complete recoveries.

SUMMARY

Thirteen cases of miliary tuberculosis and 21 cases of tuberculous meningitis were treated with a combination of streptomycin and Promizole. Eleven patients with miliary tuberculosis and 16 cases of tuberculous meningitis survive. Three patients with meningitis relapsed; one of these died.

Early recognition of the clinical picture of tuberculous meningitis and intensive intrathecal therapy are important factors in survival. The location of the lesion, the sensitivity of the microorganisms, and possibly the age of the patient at the onset of the meningitis are important in prognosis.

Combined therapy with streptomycin and Promizole, properly administered, appears to have improved the results and is worthy of more extensive clinical trial.

SUMARIO

Estreptomicino-Promizolo-Terapia de la Granulia y la Tuberculosis Meníngea en la Infancia

Trece casos de granulia y 21 de tuberculosis meníngea fueron tratados con una combinación de estreptomicina y promizol. Once de los enfermos con granulia y 16 de los de meningitis tuberculosa continúan bien. Tres enfermos con meningitis recidivaron, y uno de ellos murió.

El reconocimiento temprano del cuadro clínico de la meningitis tuberculosa y la intensa terapéutica intratecal representan importantes factores en la sobrevivencia; la localización de la lesión, la sensibilidad del microbio y posiblemente la edad del enfermito al atacar la meningitis revisten importancia en el pronóstico.

La combinación terapéutica de la estreptomicina con el promizol, debidamente administrada, parece haber mejorado los resultados y justifica pruebas clínicas más extensas.

REFERENCES

(1) Feldman, W. H., Hinshaw, H. C., and Mann, F. C.: The effect on previously established tuberculosis of guinea pigs of 4,2′-diaminophenyl-5′thiazolylsulfone (promizole), Am. Rev. Tuberc., 1944, *50*, 418.

(2) Feldman, W. H., Hinshaw, H. C., and Pfuetze, K. H.: The clinical administration of 4,2′-diaminophenyl-5′thiazolesulfone (promizole) in tuberculosis: A preliminary report, Proc. Staff Meet., Mayo Clin., 1944, *19*, 33.

(3) Milgram, L., Levitt, I., and Unna, M.: Promizole treatment of miliary tuberculosis, Am. Rev. Tuberc., 1947, *55*, 144.

(4) Lincoln, E. M., Stone, S., and Hoffman, O. R.: The treatment of miliary tuberculosis with promizole, Bull. Johns Hopkins Hosp., 1948, *82*, 56.

(5) Lincoln, E. M., and Kirmse, T. W.: Streptomycin and promizole in miliary tuberculosis and tuberculous meningitis in children, Lancet, 1949, *1*, 767.

(6) Feldman, W. H., Hinshaw, H. C., and Moses, H. E.: Promin in experimental tuberculosis, Am. Rev. Tuberc., 1942, *45*, 303.

(7) Medlar, E. M., and Sasano, K. T.: Promin in experimental tuberculosis in the guinea pig, Am. Rev. Tuberc., 1943, *47*, 618.

(8) Smith, M. I., McCloskey, W. T., and Emmart, E. W.: Influence of streptomycin and promin on proliferation of tubercle bacilli in the tissues of albino rat, Proc. Soc. Exper. Biol. & Med., 1946, *62*, 157.

(9) Callomon, F. T., Kolmer, J. A., Rule, A. M., and Paul, A. J.: Streptomycin and diasone in the treatment of experimental tuberculosis in guinea pigs, Proc. Soc. Exper. Biol. & Med., 1946, *63*, 237.

(10) Lincoln, E. M., Kirmse, T. W., and DeVito, E.: Tuberculous meningitis in children: A preliminary report of its treatment with streptomycin and promizole, J.A.M.A., 1948, *136*, 593.

(11) Woods, A. C., and Wood, R. M.: Studies in experimental ocular tuberculosis. XII. Effect of streptomycin and promizole on experimental ocular tuberculosis in the immune-allergic rabbit, Arch. Ophth., 1948, *40*, 413.

(12) Karlson, A. G,. Pfuetze, K. H., Carr, D. T., Feldman, W. H., and Hinshaw, H. C.: The effect of combined therapy with streptomycin, para-aminosalicylic acid and promin on the emergence of streptomycin-resistant strains of tubercle bacilli: A preliminary report, Proc. Staff Meet., Mayo Clin., 1949, *24*, 85.

(13) Medical Research Council.: Streptomycin treatment of tuberculous meningitis, Lancet, 1948, *1*, 582.

(14) Bunn, P. A.: One hundred cases of miliary and meningeal tuberculosis treated with streptomycin, Am. J. M. Sc., 1948, *216*, 286.

THE EFFECT OF STREPTOMYCIN UPON THE PATHOLOGY OF TUBERCULOUS MENINGITIS[1]

WILLIAM J. WINTER

(Received for publication September 26, 1949)

INTRODUCTION

The advent of streptomycin offered a new hope for the effective treatment of tuberculosis and particularly for the two most dreaded manifestations of the disease, namely, tuberculous meningitis and generalized miliary tuberculosis. Since the introduction of streptomycin in 1946, reports have been accumulating in the literature describing the successful management of the miliary disease. Tuberculous meningitis, however, has continued to remain an almost universally fatal disease. Numerous patients have lived for many months beyond the usual expectation of life in this disease, and some have had long periods of clinical remission between the onset of the disease and the final fatal relapse. Clinicians soon observed that neurological sequelae were common findings in individuals who survived the initial periods of acute meningitis and also that one or more relapses frequently occurred in the streptomycin-treated patients. These observations suggested that a full histopathological study of tuberculous meningitis as altered by streptomycin would be worth while. To this end a group of 12 fatal cases have been studied and the changes produced have been compared in detail with a control series of cases which had not had streptomycin therapy.

It soon became apparent that the problem of neurological complications was directly related to two frequent pathological findings, namely, ischemic and infective softening of the brain, and the development of hydrocephalus.

The purpose of this report, then, is to describe in some detail the pathological changes that account for the development of these frequent and undesirable complications in streptomycin-treated tuberculous meningitis.

MATERIAL AND METHODS

Material: The cases selected for study include 12 cases of meningitis treated with streptomycin for variable periods of time and in which the disease had been present for 21 to 270 days. These cases were obtained from several sources. Five were from the Hospital for Sick Children, Toronto; 4 were from the wards of the Toronto General Hospital; and 3 were from the Tuberculosis Sanitorium at Weston, Ontario.

The details of the clinical history in each of these cases were obtained from both the clinical records and the autopsy protocols. The gross description of the brain was made by personal observation in 7 cases and obtained from the autopsy protocols in the remainder. The patients differed in age (although all were white children or young adults), duration of meningitis, and the amount of treatment they received. The main clinical details are outlined in table 1.

For the control series 30 cases were selected from the autopsy records of the Division of Neuropathology, University of Toronto. The majority of this group died within two to

[1] From Division of Neuropathology, Department of Pathology, University of Toronto.

three weeks from the onset of their meningitis, although a few patients survived as long as two months.

Methods: All the brains were fixed by suspension in 10 per cent formol-saline for periods of one to two weeks. Selected blocks were embedded in paraffin. The microscopic sections were routinely stained by the following techniques:

1. Hematoxylin and eosin stain
2. Mallory's connective tissue stain
3. Mallory's phosphotungstic acid hematoxylin stain
4. Cresyl violet stain

TABLE 1

Composite Chart Outlining the Age Distribution, Duration of Disease, Treatment and Main Pathological Manifestations of the Disease

CASE	AGE	DURATION OF MENINGITIS	STREPTOMYCIN TOTAL DOSAGE		LENGTH OF THERAPY	CEREBRAL COMPLICATIONS	ASSOCIATED TUBERCULOSIS
			I.M.	I.T.			
		days	*Gm.*	*Gm.*	*days*		
P. D.	6	270	152	8.4	154*	Hydrocephalus	Caseous hilar lymph nodes
D. C.	19	225	246	3.1	110*	Hydrocephalus	Obsolete pulmonary
E. M.	25	123	450†	1.8	90‡	Hydrocephalus and softening	Healing miliary
J. LeS.	10	105	96	1.0	86	Hydrocephalus and softening	Healing miliary; caseous hilar lymph nodes
L. N.	2	103	88	0.75	88	Hydrocephalus and softening	Pott's disease; healing miliary in lungs
W. O.	30	74	114.8	3.5	46		Fibrocaseous pulmonary; healing miliary
J. M.	12	60	28	0.15	30	Softening	Caseous hilar lymph nodes; healing miliary lesions
D. S.	17	59	47	4.6	45	Softening	Healing miliary
R. D.	2	40	9.0	2.25	10	Softening	Pulmonary
L. M.	15	35	90	3.0	30	Softening	Pulmonary fibrocaseous; Pott's disease
D. W.	17	28	2.0	0.4	4	Softening	Active miliary
B. C.	20	21	0.6	0.6	12	Softening	Pulmonary

* Interrupted treatment.

† Intramuscular therapy for preceding miliary tuberculosis.

‡ Intrathecal therapy only.

When indicated by findings with the routine staining methods, additional sections were stained by Laidlaw's method for reticulum fibers, Smith and Quigley's method for myelin sheaths, and Ziehl-Neelsen's preparations were made for acid-fast bacilli.

OBSERVATIONS

Comprehensive Summary of the Untreated Cases

For the sake of a comparative review, a composite description of the histopathological findings in the untreated cases of tuberculous meningitis in the control

series will be given, preliminary to the detailed study of the treated cases. In the acute untreated cases of two to six weeks' duration, tuberculous inflammation in the subarachnoid space has a fairly uniform appearance. A narrow pial zone of lymphocytes and amoeboid acidophilic histiocytes is usually interrupted by multiple foci of necrosis. The outer zone extending to the arachnoid is broader, although its width depends upon the region of the subarachnoid space that is studied. It is composed, however, of a loose serofibrinous mesh in which there are considerable numbers of inflammatory cells, a large proportion of which are polymorphonuclear leukocytes. As a rule, many of these cells are degenerating and usually contain prominent foci of necrosis, often in perivascular situations.

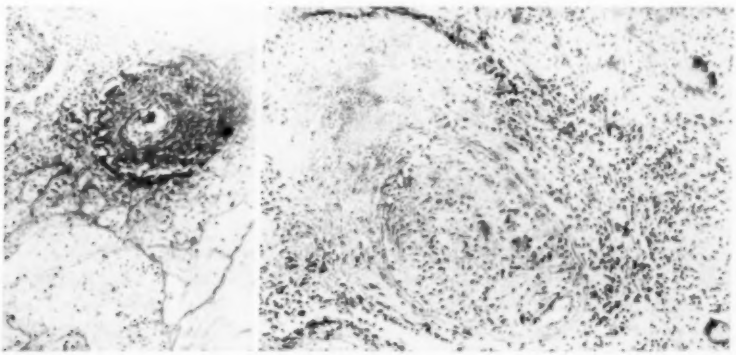


FIG. 1. (Left) Non-streptomycin-treated tuberculous meningitis showing acute inflammation of the adventitia and subendothelial layer of a meningeal artery. (Hematoxylin and eosin × 65).

FIG. 2. (Right) Non-streptomycin-treated tuberculous meningitis showing severe intimal proliferation in a meningeal artery opposite an area of caseation necrosis. (Hematoxylin and eosin × 150).

The smaller arteries almost uniformly show acute inflammation and fibrinoid necrosis (figure 1). In the absence of necrosis, the adventitia of these vessels is infiltrated with histiocytes and leukocytes and shows a proliferation of fibroblasts. The vessel wall is variably infiltrated with inflammatory cells, but the striking lesion is the marked subendothelial edema and cellular infiltration producing severe stenosis of the vessel's lumen. Fibrinoid masses are commonly seen in the subendothelial layer. Phlebitis is a common accompanying lesion to the arteritis. Ischemic softening of the brain was observed in only one of the 30 cases and occurred in the basal ganglia.

In marked contrast to this acute tuberculous inflammation is the chronic process seen in the localized meningitis about old cortical or subarachnoid tubercles. Fibrosis, often about foci of caseous necrosis, is the prominent feature. Usually both the pia mater and arachnoid are thickened and adherent. The inflammatory cells are predominantly lymphocytes with epithelioid histiocytes

forming the inner cellular wall of the tubercle. Since such tubercles are usually in juxtaposition to small arteries, these vessels may show a very striking proliferative intimal fibrosis associated with chronic inflammatory and degenerative changes in the media (figure 2). Usually such intimal disease is in the form of crescentic plaques, but occasionally it may be diffuse and produce marked stenosis of the lumen and possible focal softening of the part of the brain supplied by the vessel.

Histological Findings in Streptomycin-treated Cases

The pathological changes found in the streptomycin-treated cases of meningitis have been summarized in some detail according to the anatomical structures involved, namely: the meninges and subarachnoid space, the cerebral blood vessels, the cerebral substance, and the ependymal lining of the ventricles. An effort has been made to describe the development of the lesions in the various anatomical situations in some degree of chronological progression, although it must be realized that the rate of progression as well as the extent and severity of the changes were variable from case to case. The changes were also subject to alterations that were undoubtedly the result of such factors as dosage of the drug, duration of treatment, susceptibility of the microorganisms, and the interval elapsing between the onset of the meningitis and the institution of therapy. Natural immunity presumably also played a role. Such factors are obviously difficult to evaluate in their individual or collective effects upon the healing and resolution processes of the individual case.

The meninges and subarachnoid space: Since the most extensive exudate in tuberculous meningitis occurs in the basal cisterns of the brain, the changes were studied to best advantage in these sites. The earliest changes showed a disappearance and dissolution of the polymorphonuclear leukocytes from the exudate as early as eleven and twelve days after the institution of streptomycin, although such cells were still present in large numbers in untreated cases of comparable duration. The fibrin component was still abundant, although liquefaction was noted. Lymphocytes and histiocytes accumulated in the pial zone, and after thirty days of treatment small tubercles containing Langhans' giant cells had formed. Coincident with the accumulation of cells in the pial zone, a thickening of this membrane by fibroblastic proliferation occurred with the formation of coarse reticulum and collagenous fibers. The arachnoid also showed a similar fibrous proliferation and such changes were observed as early as eleven days after institution of treatment. As the fibrous thickening of these membranes progressed, a proliferation of fine capillaries ensued resulting in a typical tuberculous granulation tissue. The subarachnoid space, therefore, tended to become obliterated except for occasional lakes of serum or fibrin and islands of caseation necrosis. In the patients who had lived for three or four months without true clinical remission, the fibrosis assumed dense proportions, although tending to be more intense in some places than in others. In this respect dense collagenous fibrosis was more marked in the deep sulci of the basal convolutions and the lateral fissures than in the wide spaces of the basal cisterns (figure 3). Although

in several instances of long duration caseous lesions were found with a well-defined fibrous capsule, it was not uncommon to find caseating areas which were partly infiltrated by radially arranged fibroblasts similar to the healing process which has been described in miliary tubercles in other organs of the body (1, 2), (figure 4). It is of interest that in one case showing encapsulated caseous tubercles in a deep sulcus, the surrounding dense fibrous tissue merged into a more acute exudate containing fibrin and numerous inflammatory cells at the mouth of the sulcus.

Lesions that were to all intents and purposes obsolete tubercles have been found in the spinal subarachnoid space (figure 5). Such tubercles were relatively acellular and almost completely hyalinized. Acid-fast bacilli could not be found by Ziehl-Neelsen stains. Since they occurred in the lumbar region where the intrathecally administered streptomycin would be present in largest concentration with repeated doses, it is likely that such lesions represent the maximum healing response of the diseased tissues.

Cerebral blood vessels: The vascular inflammatory changes observed in streptomycin-treated tuberculous meningitis are distinctly progressive. As is so characteristic of tuberculous meningitis, the small arteries were most severely damaged, although the large-caliber vessels did not escape. The earliest changes were acute exudation in the intima such as was seen in the untreated cases, but proliferation of subendothelial fibroblasts occurred as early as eleven days after the institution of treatment. Fibrinoid necrosis was still seen in some vessels, in one case even after a month of treatment. Gradually, however, the inflamed swollen subendothelial tissues became filled in with fibroblasts, although the deposition of collagen fibrils was not apparent before forty-five to sixty days of treatment (figure 6). The intimal fibrosis was frequently concentric, but in numerous instances it tended to be thicker on the side of the vessel in apposition to a meningeal tubercle or focus of caseation. All degrees of stenosis thus occurred. Virtual obliteration of the lumen was observed in some arteries, the structure of which was so altered by tuberculous granulation tissue that they were recognizable as such only by the persistence of the internal elastic lamina. Some such vessels not uncommonly showed typical giant cells formed within the degenerated media or hyperplastic intima (figure 7). Occasionally all that remained of a lumen were small recanalizing capillary channels hardly sufficient to maintain an adequate circulation. Such severe lesions were found in the cases of three to four months' duration with extensively organized meningeal exudate. Thrombosis was not observed in any of the inflamed vessels in this series.

In contradistinction to the relative uniformity of the arteritis in a given area of meningitis in the untreated cases, such uniformity was not the rule in the streptomycin-treated cases. The greater number of small arteries in an area, such as the basal cisterns, usually showed similar progressive changes, but at the same time other vessels of similar caliber were relatively intact. Moreover, all the diseased vessels in such a region did not necessarily show the same degree of inflammatory changes. Whereas some showed chronic proliferative lesions, others in the immediate area were in a stage of acute inflammation. Generally, however,

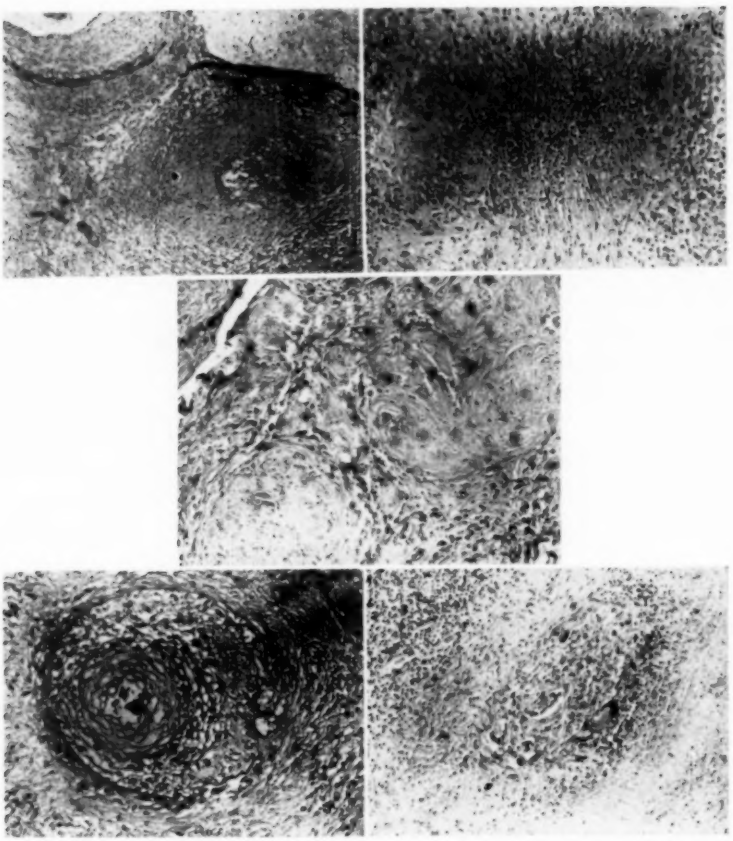


FIG. 3. (Upper left) Streptomycin-treated tuberculous meningitis of nine months' duration showing marked fibrous organization of the subarachnoid exudate and encapsulation of a caseous lesion. (Hematoxylin and eosin × 50).

FIG. 4. (Upper right) Streptomycin-treated tuberculous meningitis of three months' duration showing infiltration of a necrotic area by radially-arranged fibroblasts. (Hematoxylin and eosin × 110).

FIG. 5. (Center) Streptomycin-treated case of seven and one-half months' duration showing a healing fibrous organization of tuberculous meningitis in the lumbar subarachnoid space. (Hematoxylin and eosin × 180).

FIG. 6. (Lower left) Streptomycin-treated tuberculous meningitis of forty-five days' duration showing severe subendothelial fibroblastic proliferation of a meningeal artery. Inflammatory cells lie among the concentric fibrils of the fibroblasts. (Hematoxylin and eosin × 110).

FIG. 7. (Lower right) Streptomycin-treated tuberculous meningitis of three and one-half months' duration showing almost complete obliteration of the lumen of a meningeal artery by subendothelial fibroblastic proliferation. The media is degenerate l. (Hematoxylin and eosin × 110).

the stage of arteritis was comparable to the acuteness or chronicity of the inflammation in the adjacent meningeal tissue.

The arteries of large caliber escaped the severe stenosing inflammatory changes that were so marked in the small vessels. Uniformly, however, such arteries as the middle cerebral and basilar showed minor degrees of inflammation. The intimal lesions consisted of fibroblastic proliferation along one side of the vessel which showed an adventitial tuberculous focus. Medial degeneration and inflammation were usually minimal. In no instance was the lumen of any of these vessels found unduly narrowed. The formation of such intimal fibrosis is an early phenomenon and was observed in a 2-year-old child with meningitis of forty days' duration who had had only ten days of streptomycin therapy.

Changes in the brain tissue: Nine of the 12 cases showed areas of ischemic softening which were directly related to the progressive tuberculous arteritis. Grossly these lesions were found in the basal ganglia and internal capsule in every instance in which softening occurred. When the meningitis was of short duration, these sites were usually the only areas to be found, but in the more advanced disease which had been present for three to four months before death softening had occurred in other anatomical sites as well. In only one brain was the softening of the basal ganglia unilateral (figure 8). In all the others it had occurred on both sides, although not always to an equal extent. Although the basal ganglia were the most common sites for softening, the other areas included local softenings in the temporal poles, foci in the mid-brain and hypothalamus, and both focal and massive areas in the cerebral hemispheres along the distributions of branches of the anterior, middle, and posterior cerebral arteries. In gross appearance these lesions varied from soft, swollen, pallid areas to actual cystic degeneration. Some of the brains also showed severe edema of the white matter associated with the degenerative lesions. In one child who had repeated convulsions a few days prior to death, edema was extraordinarily severe.

The histological lesions varied with the duration of the disease. The earliest lesions showed severe perivascular and pericellular edema associated with nerve cell degeneration and early demyelination. In one instance the softening was not of more than forty-eight hours' duration. This patient had been treated with streptomycin for only four days, although her meningeal symptoms had been present for nearly a month. She had multiple softenings but all of these had the same histological appearance. The coincidence of streptomycin therapy and brain softening in this case is curious. Another early case showed, in addition to the nerve cell and myelin degeneration, a striking proliferation of astrocytes chiefly in the white matter. Much older lesions, of weeks' or several months' duration, were seen in the remainder of the brains. In these lesions the nerve cells and fibers had disappeared and were replaced by swarms of cerebral histiocytes (figure 9). An occasional area, however, showed a pronounced proliferation of piloid astrocytes forming a bridging network through the degenerated tissue (figure 10).

Infective softening played a minor role in the production of neurological complications. Commonly the floor of the third ventricle and subpial zone of the brain stem showed inward extension of the tuberculous inflammation from the

subarachnoid space. In several instances microscopic tubercles were found along the course of penetrating vessels or tuberculous areas of inflammation, and necrosis occurred in the subependymal regions of the basal ganglia and thalamus. Similar microscopic lesions were occasionally found in the substance of the pons

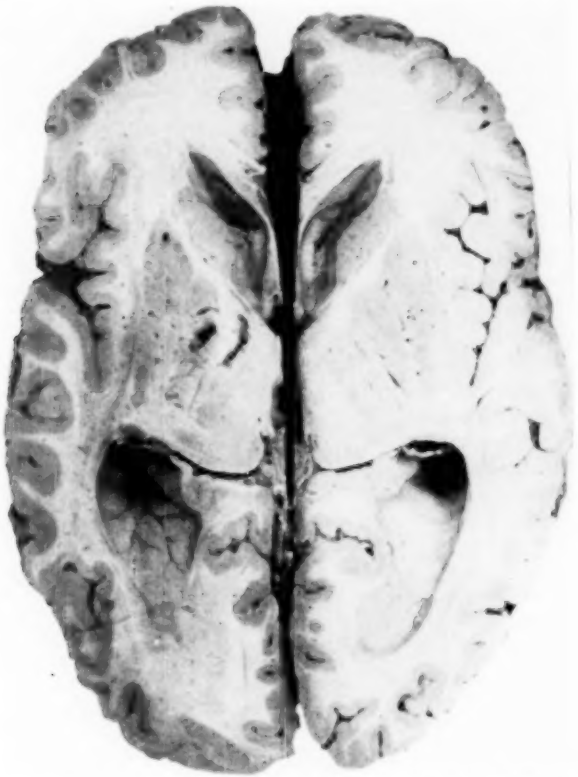
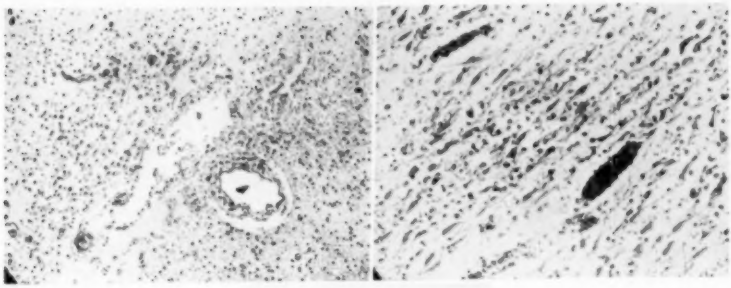


FIG. 8. Streptomycin-treated case of forty-five days' duration. Horizontal section of cerebral hemispheres showing cystic softening involving the left globus pallidus and genu of the internal capsule. This lesion is the result of severe tuberculous endarteritis in the interpeduncular fossa and lateral fissure. (Two-thirds normal size).

and mid-brain. Focal death of nerve cells and demyelination were associated with such lesions. Such tuberculous lesions usually did not show the same degree of healing or organization that was seen in the meninges. A rather marked astrocyte response was noted, however, in the subpial zones of the base of the brain beginning even in the earliest cases.

The ependymal lining of the ventricles: Infection of the ependyma had occurred in all of the brains studied. The changes included those in the ependymal lining



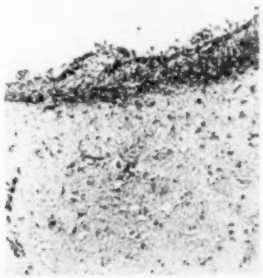


Fig. 9. (Upper left) Streptomycin-treated tuberculous meningitis of forty-five days' duration showing beginning cystic softening of the globus pallidus and diffuse infiltration by cerebral histiocytes. The section corresponds to the lesion shown in figure 8. (Hematoxylin and eosin × 66).

Fig. 10. (Upper right) Streptomycin-treated tuberculous meningitis of three and one-half months' duration showing proliferation and parallel arrangement of piloid astrocytes in an area of cystic softening in the head of the caudate nucleus. (Hematoxylin and eosin × 130).

Fig. 11. (Bottom) Streptomycin-treated tuberculous meningitis of three and one-half months' duration showing subependymal gliosis in the wall of a lateral ventricle with protrusion of a tuft of glial cells. (Hematoxylin and eosin × 110).

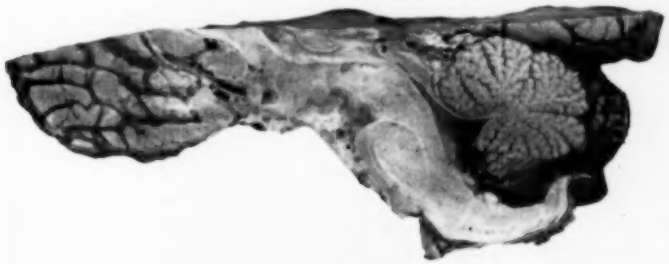


Fig. 12. Streptomycin-treated case of forty-five days' duration. Sagittal section of right half of brain showing extensive granulomatous thickening in the interpeduncular fossa and miliary tubercles over the medial surface of the right frontal lobe. This photograph shows congested vessels firmly embedded in the tuberculous exudate. Granular ependymitis is seen in the roof of the fourth ventricle. (Two-thirds normal size).

cells and in the subependymal tissues. When a relatively acute tuberculous infection was present, the ependymal cells were swollen and often desquamated in company with histiocytes and a few lymphocytes over the site of a subependymal tuberculous nidus. Such subependymal lesions consisted of large or small loosely composed aggregations of histiocytes, some lymphocytes, and sometimes bizarre swollen astrocytes. Usually a local or diffuse edema of this zone was also present. The local vessels were commonly encircled by distinct cuffs of lymphocytes and histiocytes. As healing took place, the subependymal inflammation showed the formation of distinct tubercles and the intervening tissue became markedly gliosed. A dense compact layer of glial fibers could be demonstrated with Mallory's phosphotungstic acid stain. The overlying ependymal epithelium appeared more uniform and cuboidal, although occasionally small hyperplastic plaques of ependymal cells two or three layers thick were found. In keeping with the gross granularity of the ependymal surface, which was observed even after the subsidence of the acute ependymitis, the lining cells were disrupted in numerous places by an extrusion of glial cells with small tufts of their fibers into the ventricle, such as is seen in the ependymitis of general paresis (figure 11).

DISCUSSION

The end results of streptomycin treatment of tuberculous meningitis in terms of survival are disheartening even though they are still remarkable in comparison with pre-streptomycin days. It appears that streptomycin-treated tuberculous meningitis may pursue one of several courses. There may be a steady deterioration even with marked prolongation of life but little, if any, clinical improvement. Such cases rarely live more than two or three months. Progressive deterioration may occur after a short initial period of improvement or a fatal relapse may appear after a long period of improvement. Improvement may be uninterrupted from almost the very onset of streptomycin treatment and occasionally, but rarely, improvement may occur after a relapse.

The cases in this series, which represent variations in age of the patient as well as in the type of institutional case, fall into three fairly distinct groups with reference to their mode of death. Arbitrarily these cases may be classified as: acute, including the group which died within two months and showed no clinical improvement; subacute, including those patients who lived three to four months with at least a short interval of improvement; and the chronic group with a definite clinical remission between the initial disease and the fatal relapse. In the acute cases, at the time of death the meningeal inflammation varied from a fibrinonecrotic exudate containing numerous polymorphonuclear leukocytes and showing little, if any, fibroblastic proliferation to early or moderate formation of granulation tissue. The granulation tissue extended from both the pia mater and arachnoid with a varying amount of fibrinonecrotic material in the intervening subarachnoid space. There was a similar variation in the inflammation of the small arteries. In all of this group of cases, early ischemic softening had occurred in the basal ganglia and sometimes had progressed to cystic degeneration. The brain was always severely edematous and in one 2-year-old boy

it weighed 1,625 grams. There was no significant hydrocephalus. All of these patients died with the clinical picture of toxemia associated with increased intracranial pressure. All died in coma which had persisted for several days to two or three weeks; one had numerous convulsions for three days before death, and 4 had developed a terminal decerebrate rigidity.

There were 3 patients with a subacute type of disease who had lived from three to four months after the onset of their meningitis. Two of these had shown a marked clinical improvement for several weeks before the final decline. In each case hydrocephalus was severe at the time of death and was directly related to the terminal symptoms of persistent vomiting, generalized spasticity of the limbs, dilated pupils, and coma. The meningeal exudate in these brains showed extensive fibrous organization which could certainly be expected to prevent any circulation of the cerebrospinal fluid from the posterior fossa to the supratentorial cisterns. The tuberculous arteritis was almost entirely a proliferative form with severe fibroblastic thickening of the intima of the small arteries. An occasional vessel was obliterated by granulation tissue. Although the larger arteries showed eccentric intimal plaques of fibrous tissue, no occlusions were seen, such as Smith *et al.* found in 2 cases by arteriography (4). Foci of ischemic softening had occurred in the basal ganglia as well as in other areas of the brain. Tubercle bacilli were consistently found, although few in number.

The 2 cases which constitute the third group of chronic meningitis each had prolonged periods of remission followed by fatal relapse. Severe hydrocephalus due to exudative blocking of the cerebello-medullary foramina in one case and the incisura tentorium in the other was found at autopsy. The brains of both of these individuals showed distinct old tuberculous lesions, even to caseation and encapsulation in deep sulci of the cerebral and cerebellar hemispheres. One patient had developed a spinal block at the site of well-organized healing meningitis, and, interestingly enough, the bacilli in this case were sensitive *in vitro* to the same concentration of streptomycin as those isolated at the initial infection. This has been a common experience in other reports of patients who have died after a long remission (5). The acute nature of the exudate at the base of the brain and the unremitting deterioration of the patient during his relapse also suggests that streptomycin administered intrathecally at the lumbar level had failed to reach the infection in the cranium.

It is readily apparent that streptomycin treatment of tuberculous meningitis has been only the opening page for the effective treatment and cure of the disease. It is not yet the answer, but it has made the problems of treatment more clearly apparent. The effective therapeutic agent must not only be highly lethal to the tubercle bacilli and almost instantaneously so, but it must itself or by effective combination with some other agent rapidly dissipate the fibrin exudate and retard the formation of fibrous tissue. Recently the use of a fibrinolytic enzyme (streptokinase) elaborated by *Streptococcus* has been reported from England with encouraging results (3). It has also been a not uncommon practice to combine heparin with intrathecally administered streptomycin. The problem of the prevention of hydrocephalus remains of prime importance.

The problem of ischemic brain softening is of less clinical importance than hydrocephalus. When infarcts of the basal ganglia have been severe, the patients succumbed either to an acute unremitting infection which streptomycin affected but little, or else they died with severe hydrocephalus due to organization of the subarachnoid exudate. There has been no opportunity as yet to observe old softenings in patients who have had long remissions with partial recovery from neurological symptoms due to such lesions. There have been reports in the literature of remarkable recovery from various neurological lesions, such as hemiplegia, and it is fair to assume that, in the patient showing progressive improvement in the meningitic complex, the ischemic lesions will probably have been relatively small and heal to small cystic cavities which leave little, if any, residual neurological signs (5).

SUMMARY

1. Twelve fatal cases of streptomycin-treated tuberculous meningitis were studied histologically with emphasis upon the pathological changes occurring in the leptomeninges, meningeal arteries, brain substance, and ependymal lining of the ventricles.

2. The cases fell into three groups: first, the acute meningitis in which death occurred within two months without evidence of clinical improvement; second, subacute disease in which the patient lived three to four months with short intervals of improvement; and third, chronic disease in which there had been good clinical remissions for several months.

3. Streptomycin induces a definite reparative process in tuberculous meningitis, as shown by the prolongation of life and the pathological changes observed in these fatal cases. These apparently occur as follows:

The exudate in the subarachnoid space undergoes progressive organization. There is early dissolution of much of the fibrin and replacement by actively proliferating fibroblasts and chronic inflammatory cells. The foci of necrosis tend to be either encapsulated or partially infiltrated by fibroblasts.

The acute tuberculous arteritis, commonly involving the vessels in the Circle of Willis and proximal branches of the middle cerebral arteries, rapidly is transformed into a proliferative endarterial reaction causing severe stenosis or occlusion of the vessels.

Multiple ischemic softenings, which are consistent in the basal ganglia and internal capsule, result from the endarteritis, and are a common cause of neurological sequelae.

Tuberculous ependymitis may resolve with the formation of small subependymal tubercles and well marked subependymal gliosis. Persistent granularity of the ependymal lining in a chronic case may be due to gliosis with the inward projection of numerous small glial tufts.

4. The outstanding complications of streptomycin-treated tuberculous meningitis are communicating hydrocephalus and multiple ischemic softenings of the brain.

5. Spinal block may develop due to extensive fibrous organization of the

meningeal exudate in the vicinity of the site of intrathecal injections of the streptomycin.

SUMARIO

El Efecto de la Estreptomicina sobre la Anatomía Patológica de la Meningitis Tuberculosa

1. Doce casos letales de meningitis tuberculosa tratada con estreptomicina fueron estudiados histológicamente, haciendo hincapié en las alteraciones patológicas observadas en las leptomeninges, arterias meníngeas, sustancia cerebral y membrana ependimaria de los ventrículos.

2. Los casos correspondían a tres grupos: primero, meningitis aguda en la cual la muerte sobrevino en término de dos meses sin signos de mejoría clínica; segundo, casos agudos que vivieron de tres a cuatro meses con breves intervalos de mejoría; y tercero, la afección crónica en la que hubo buenas remisiones clínicas por espacio de varios meses.

3. La estreptomicina evoca en la meningitis tuberculosa un proceso regenerativo bien definido según patentizan la prolongación de la vida y las alteraciones patológicas observadas en esos casos letales.

El exudado en el espacio subaracnoideo experimenta organización progresiva. Hay disolución temprana de gran parte de la fibrina y sustitución por fibroblastos en proliferación activa y células en inflamación crónica. Los focos de necrosis suelen hallarse encapsulados o parcialmente infiltrados por fibroblastos.

La arteritis tuberculosa aguda, que afecta habitualmente los vasos del círculo de Willis y las ramas proximales de las arterias cerebrales medias, transfórmase rápidamente en una reacción endarterial proliferativa que ocasiona grave estenosis u oclusión de los vasos.

Los reblandecimientos isquémicos múltiples, que son constantes en los ganglios basales y la cápsula interna, provienen de la endarteritis y constituyen una causa común de secuelas neurológicas.

La ependimitis tuberculosa puede resolverse con la formación de pequeños tubérculos subependimarios y pronunciada gliosis subependimaria. La persistente granulación de la membrana ependimaria en un caso crónico puede deberse a gliosis con proyección interna de numerosos glomerulillos de neuroglia.

4. Las complicaciones más notables de la meningitis tuberculosa tratada con estreptomicina son hidrocefalia externa y reblandecimientos isquémicos múltiples del cerebro.

5. Puede sobrevenir bloqueo raquídeo debido a la extensa organización fibrosa del exudado meníngeo en la vecindad del sitio de las inyecciones intratecales de la estreptomicina.

Acknowledgment

The writer wishes to acknowledge the help and advice given him in the preparation of this paper by Dr. Eric A. Linell, Professor of Neuropathology, University of Toronto, and Dr. George H. Fetterman, Chief Consultant in Pathology, Veterans Administration Hospital, Aspinwall 15, Pennsylvania. He also wishes to acknowledge the cooperation of Dr.

William S. Donahue, Pathologist, Sick Children's Hospital, Toronto, and Dr. W. Anderson, Pathologist, Toronto Tuberculosis Sanitorium, Weston.

REFERENCES

(1) Baggenstoss, A. H., Feldman, W. H., and Hinshaw, H. C.: Streptomycin in miliary tuberculosis, Am. Rev. Tubere., 1947, *55*, 54.

(2) Wright, G. P.: Histology of typical lesions after streptomycin, Lancet, December 20, 1947, *253*, 903.

(3) Bunn, P. A.: One hundred cases of miliary and meningeal tuberculosis treated with streptomycin, Am. J. M. Sc. 1948, *216*, 286.

(4) McDermott, W., Muschenheim, C., Hadley, S. J. Bunn, P. A., and Gorman, R. V.: Streptomycin in the treatment of tuberculosis in humans: I. Meningitis and generalized hematogenous tuberculosis, Ann. Int. Med., 1947, *27*, 769.

(5) Smith, H. V., Vollum, R. L., and Cairns, H.: Treatment of tuberculous meningitis with streptomycin, Lancet, April 24, 1948, *1*, 627.

(6) Medical Research Council: Streptomycin treatment of tuberculous meningitis, Lancet, April 17, 1948, *1*, 582.

(7) Cathie, I. A. B.: Streptomycin-streptokinase treatment of tuberculous meningitis, Lancet, March 12, 1949, 1, 411.

(8) Madigan, D. G., and Swift, P. N.: Treatment of tuberculosis with streptomycin and sulphetrone, Lancet, December 20, 1947, *253*, 837.

(9) Mehas, C. P., and Truax, W. E.: Streptomycin in tuberculous meningitis, J. A. M. A., 1947, *135*, 155.

TUBERCULOUS BRONCHITIS IN PULMONARY RESECTION[1]

Incidence in Fifty-eight Pulmonary Resections for Tuberculosis

MAURICE G. BUCKLES AND WILFORD B. NEPTUNE

(Received for publication May 3, 1949)

Fifty-eight cases of pulmonary resection for tuberculosis have been performed by the senior author (M.G.B.) at the Franklin County Tuberculosis Hospital. In reviewing the surgical pathology of the resected specimens, a marked difference was noted between tuberculous endobronchial disease diagnosed by bronchoscopy as compared to its demonstration by the pathologist. The difference was so significant that it was believed that the findings should be reported.

CLINICAL MATERIAL

Fifty-eight operations were performed on 57 patients. Six Negroes were included in the group of 27 men and 30 women. The ages varied from eighteen to fifty, with an average age of thirty-two years.

According to the National Tuberculosis Association classification, 3 minimal, 17 moderately advanced, and 37 far advanced cases of tuberculosis were included in the series, and the various types of cases were evenly distributed between the sexes (table 1).

The patients had been known to have pulmonary tuberculosis from three months to fifteen years previous to surgery, with an average time interval of 3.8 years. Sixteen patients had had their disease for more than five years and 8 for more than ten years. In the entire group, 14 had suffered from one to two relapses during the course of their disease.

Only 5 patients had received no collapse therapy previous to pulmonary resection, and pneumothorax had been attempted in 3 of these. The previous type of collapse therapy in the group is listed in table 2. Most of the phrenic nerve crushes on the homolateral lung were in conjunction with pneumoperitoneum or pneumothorax. Only those patients with pneumothorax in whom the procedure was given a trial of four months or longer are listed.

SURGICAL MATERIAL

All patients in the hospital receive a diagnostic bronchoscopy prior to any type of collapse therapy. If a tuberculous endobronchial lesion is demonstrated, the patient is treated accordingly. All patients receive a diagnostic bronchoscopy just prior to major thoracic surgery. At the present time, if an active tuberculous endobronchial lesion is demonstrated, the patient receives a course of streptomycin and surgical treatment is deferred until re-examination reveals healing of the lesion. The results in tracheobronchial tuberculosis treated with streptomycin in this clinic have been similar to those reported by others (1, 2, 3, 4).

[1] From the Department of Surgery, Ohio State University, Columbus, Ohio, and Franklin County Tuberculosis Hospital, Columbus, Ohio.

Reports in the literature concerning the incidence of tuberculous tracheobronchitis diagnosed by bronchoscopy vary considerably, depending upon whether unselected or suspicious cases are examined. Judd (5) reports an incidence of 36.8 per cent of endobronchial disease among 500 consecutive bronchoscopies. Curreri *et al.* (6) in 39 cases of pulmonary resection report a preoperative diagnosis of bronchiectasis in 12, bronchostenosis in 11, and tuberculous bronchitis in 16. Overholt *et al.* (7) in 192 patients undergoing pulmonary resection report a preoperative incidence of tuberculous bronchitis of 35 per cent. Among

TABLE 1

NTA Classification of 57 Patients in Whom Pulmonary Resection was Performed

CLASSIFICATION	MALE	FEMALE	TOTAL
Minimal	1	2	3
Moderately advanced	9	8	17
Far advanced	17	20	37

TABLE 2

Collapse Therapy on 57 Patients prior to Pulmonary Resection

I. Homolateral lung	
1. Phrenic nerve crush	28
2. Pneumoperitoneum	17
3. Pneumothorax	24
a. Closed pneumolysis	5
b. Open pneumolysis	1
4. Thoracoplasty	9
a. Monaldi	1
II. Contralateral lung	
1. Phrenic nerve crush	6
2. Pneumothorax	8
a. Closed pneumolysis	1
b. Open pneumolysis	1

the numerous reports on the subject, McIndoe, Steele, Samson, Anderson, and Leslie (8) and Buckles (18) were perhaps the first to make a systematic bronchoscopic examination of nonselected cases of pulmonary tuberculosis. In 272 routine admissions, McIndoe *et al.* found tuberculous tracheobronchitis in 11 per cent, a figure that has been approached by nearly all subsequent reports on unselected cases of pulmonary tuberculosis. It is probable that most figures over 15 per cent include cases selected because of some suspicious clinical signs (9).

Eight (14 per cent) of our 57 cases were diagnosed by bronchoscopy as having tuberculous tracheobronchitis. The criteria used for the diagnosis of such a lesion was the presence of one, or a combination, of the following:

1. a definite proliferative lesion
2. a definite ulcerative lesion
3. cicatricial stenosis

It is not believed that superficial hyperemia constitutes sufficient evidence for diagnosis by bronchoscopy. In table 3 is a résumé of the 8 cases with tuberculous tracheobronchitis diagnosed preoperatively and the treatment instituted prior to surgery.

All patients before pulmonary resection had a diagnostic bronchogram of the side to be operated, and usually a bilateral examination was made. In this group of 57 patients, bronchiectasis was diagnosed in 39 (66.6 per cent) by preoperative bronchography.

In addition to the usual preoperative procedures, all of these patients received one-week preoperative therapy with streptomycin (1.0 to 2.0 Gm. daily) and

TABLE 3

Endobronchial Disease Diagnosed by Bronchoscopy in 57 Patients

CASE LESION	TREATMENT PRIOR TO SURGERY	ASSOCIATED LESIONS
Ulcerative tracheobronchitis (3 years previous to surgery)	Silver nitrate applications	Cavity, bronchiectasis
Chronic hyperplastic laryngitis (2 years' duration)	Streptomycin 1.0 Gm. daily—6 weeks	Destroyed lung
Ulcerative tracheobronchitis	Aerosol streptomycin 1.0 Gm. daily—3 months	Bronchiectasis
Ulcerative tracheobronchitis	Streptomycin 2.0 Gm. daily—3 months	Giant cavity, bronchiectasis
Proliferative lesion, right middle lobe bronchus	Streptomycin 1.0 Gm. daily—3 months	Cavity, bronchiectasis
Proliferative lesion, right upper lobe bronchus	Streptomycin 1.0 Gm. daily—6 weeks	Cavity, bronchiectasis
Cicatricial stenosis, left common bronchus	—	Giant cavity, bronchiectasis
Cicatricial stenosis, right common bronchus	—	Giant cavity

penicillin (150,000 units daily), and this chemotherapy was continued for two weeks postoperatively or longer when indicated. Penicillin and streptomycin were placed in the pleural space at surgery and in the cases of pneumonectomy the drugs were placed in the pleural space daily for two weeks following the operation.

There were 17 pneumonectomies and 41 partial resections consisting of 27 lobectomies; 6 lobectomy and segmental resections; 6 segmental resections; and one bilateral lobectomy (tables 4 and 5).

SURGICAL PATHOLOGY

The results of the examinations[2] of the specimens obtained at operation are listed in table 6.

In the cases of chronic ulcerative tuberculosis, the size of the cavities varied from 0.5 to 11 cm. All of the bronchi were dissected and examined for gross evi-

[2] Examinations performed by Dr. H. B. Davidson, pathologist for the hospital.

dence of dilatation and the presence of endobronchial lesions. Sections were taken from all suspicious areas and from the walls of bronchi nearest the hilum

TABLE 4

RESECTION	NUMBER
Pneumonectomy	17
Partial	41
Lobectomy	27
Lobectomy and segmental	6
Segmental	6
Bilateral lobectomy	1

TABLE 5

Portion of Lung Resected in 58 Operations

Left lung	11
Right lung	6
Right upper lobe	19
Right middle lobe	10
Right lower lobe	6
Left upper lobe	9
Left lower lobe	2
Segments (10)	
Left upper lobe—apical—posterior	5
Right lower lobe—superior	3
Right middle lobe—lateral	1
Left lower lobe—superior	1
Left upper lobe—lingula	1
Right upper lobe—anterior	1
Right upper lobe—posterior	1

TABLE 6

Nature of Lesions in the Resected Pulmonary Tissue

PATHOLOGY	MALE (27)	FEMALE (30)	TOTAL (57)
Chronic ulcerative tuberculosis	21	15	36
Chronic fibrocaseous tuberculosis	5	15	20
Tuberculous bronchitis	15	23	38
Bronchiectasis	10	21	31
Nontuberculous	3	13	16
Tuberculous	7	8	15
Tuberculous pleuritis	1	0	1
Tuberculous pneumonia	2	0	2

of the surgical specimen. The incidence of tuberculous bronchitis (66.6 per cent) as reported is based on microscopic evidence. The incidence of bronchitis per se would be considerably greater.

In 1929 Minkousky (11) reported a study of 2,379 autopsies on tuberculous

patients and found 11.48 per cent of instances of tracheal tuberculosis on gross examination. Wilbur (12) reported 35.4 per cent of incidence of tuberculous bronchitis in 500 autopsies. Meissner (13) reported the finding of tuberculous bronchitis in 52 per cent of 60 specimens of lung removed by lobectomy. Sweany and Behm (9), in a recent report on 667 autopsies, found an incidence of tuberculous bronchitis of 56.5 per cent on gross examination, and the estimated incidence based on microscopic examination was 72.9 per cent. There was an incidence of 67.9 per cent in females compared with 47.4 per cent in males. A higher incidence occurred in the younger age groups with an increase as the parenchymal disease increased. There appeared to be no relation between the finding of the bronchial lesion and the presence or absence of cavity. It becomes clear that most material examined will reveal the presence of tuberculous bronchitis in over 50 per cent of the cases.

TABLE 7

Present Status of 57 Patients in Whom Pulmonary Resection Was Performed

	NUMBER	PER CENT
Clinically well with sputum negative for tubercle bacilli	27	47.4
Negative by sputum culture	27	
Negative by guinea pig inoculation	13	
Clinically well with sputum positive for tubercle bacilli	4	7.0
Positive by sputum culture only	1	
Too recent to classify (less than 4 months)	19	33.3
Dead	7	12.3

The types of disease in the patients in the present report are fairly well representative of the types of tuberculosis for which pulmonary resection has been performed, as reported in the literature. The incidence of tuberculous bronchitis as diagnosed at bronchoscopy was 14 per cent, and this compares with other reports on unselected cases. The 66.6 per cent incidence of tuberculous bronchitis, with 53.5 per cent in males and 76.6 per cent in females, as demonstrated by the pathologist, compares with reports on autopsy material.

Samson (14) clearly showed that among those patients with a diagnosis of tuberculous tracheobronchitis a fatality of 50 per cent can be expected in two to four years. It is believed that the high incidence of tuberculous bronchitis in our patients played an important part in the failure of previous collapse therapy to control the disease. It is also believed that this high incidence of tuberculous bronchitis perhaps plays an important role in the pathogenesis of "spreads" and progression of parenchymal lesions so commonly reported in the late postoperative period (7, 15, 16, 17).

PRESENT STATUS OF PATIENTS

The status of the 57 patients who were operated upon between September 1, 1947 and December 1, 1948 is presented in table 7. The 7 (12.3 per cent) patients who have expired are listed as over-all mortality and not as operative mortality. A summary of the causes of death are presented in table 8.

The sputum "conversion" to date on the 31 patients followed for more than four months is 87 per cent, and the 4 patients with sputum positive for tubercle bacilli are clinically well at present.

It is realized that a long term follow-up is necessary in any type of therapy for pulmonary tuberculosis. The sputum "conversion" here is based on negative findings in concentrate examinations of five-day pooled samples of sputum and culture of similar samples. After several negative reports have been obtained and the patient's clinical condition is satisfactory, gastric contents are inoculated into a guinea pig. All reports must be negative before sputum "conversion" is reported.

TABLE 8

Over-all Mortality of 58 Pulmonary Resections

CASE	RESECTION	DAYS POSTOPERATIVE EXPIRED	CAUSE OF DEATH (AUTOPSY)
1	Lobectomy	2	Embolus to left middle cerebral artery
2	Pneumonectomy	6	Tuberculous meningitis
3	Lobectomy	57	Pulmonary insufficiency (sputum "negative")
4	Lobectomy	60	Metastatic brain abscess, nontuberculous (sputum "negative")
5	Pneumonectomy	79	Acute contralateral spread
6	Lobectomy	92	Bronchopleural fistula; mixed tuberculous empyema
7	Pneumonectomy	93	Bronchopleural fistula; mixed tuberculous empyema; contralateral spread

SUMMARY

1. A preliminary survey of 58 pulmonary resections for tuberculosis among 57 patients is presented.

2. The preoperative diagnosis of tuberculous endobronchial disease was 14 per cent, and compares with other reports on nonselected cases. The presence of tuberculous bronchitis, as demonstrated microscopically by the pathologist, was 66.6 per cent, with 55.5 per cent in males and 76.6 per cent in females. This compares with the incidence as based on autopsy material of tuberculous patients reported in the literature.

3. It is believed that the high incidence of tuberculous bronchitis accounted for many of the failures of collapse therapy among these patients prior to pulmonary resections.

4. It is further believed that this high incidence of tuberculous bronchitis may explain the pathogenesis of "spreads" and progression of parenchymal disease in the late postoperative period so characteristically present in follow-up reports on pulmonary resection for tuberculosis. It remains to be seen how streptomycin, apparently so specific in treating tuberculous bronchitis, alters this late follow-up period.

5. The over-all mortality to date in 58 resections is 12.3 per cent. The sputum "conversion" of 31 patients followed from four to fifteen months is 87 per cent.

6. Based on the National Tuberculosis Association classification, the incidence of tuberculous invasion of the bronchial wall showed no relationship to the extent of the pulmonary disease.

SUMARIO

La Bronquitis Tuberculosa en la Resección Pulmonar

1. Preséntase un estudio preliminar de 58 resecciones pulmonares por tuberculosis en 57 enfermos.

2. El diagnóstico preoperatorio de endobronquiopatía tuberculosa representó 14 por ciento, comparándose con las cifras dadas en otras comunicaciones sobre casos tomados al azar. La presencia de bronquitis tuberculosa, comprobada microscópicamente por el patólogo, alcanzó 66.6 por ciento: 55.5 por ciento en los varones y 76.6 por ciento en las mujeres. Esto se compara bastante bien con la incidencia basada en material autópsico de tuberculosos que aparece en la literatura.

3. Parece que la elevada incidencia de bronquitis tuberculosa explica muchos de los fracasos de la colapsoterapia en estos pacientes antes de hacerse las resecciones pulmonares.

4. Parece también que esa alta incidencia de bronquitis tuberculosa puede explicar la patogenia de las difusiones y agravación de la enfermedad parenquimática en el período postoperatorio tardío, tan típicamente presente en los informes tardíos sobre la resección pulmonar por tuberculosis.

5. La mortalidad global hasta la fecha en 58 resecciones es de 12.3 por ciento. El viraje del esputo en 31 enfermos seguidos de cuatro a quince meses representa 87 por ciento.

6. A base de la clasificación de la Asociación Nacional contra la Tuberculosis, la incidencia de invasión tuberculosa de la pared bronquial no guardó relación con la extensión de la enfermedad.

REFERENCES

(1) Hinshaw, H. C., Feldman, W. H., and Pfuetze, K. H.: Treatment of tuberculosis with streptomycin, J.A.M.A., 1946, *132*, 778.

(2) Brewer, L. A., III, and Bogen, E.: Streptomycin in tuberculous tracheobronchitis, Am. Rev. Tubere., 1947, *56*, 408.

(3) Howlett, K. S., Jr., and O'Connor, J. B.: Treatment of tuberculosis with streptomycin, Am. Rev. Tubere., 1948, *58*, 172.

(4) Bunn, P. A.: Streptomycin in the treatment of human tuberculosis, Dis. of Chest, 1948, *14*, 670.

(5) Judd, A. R.: Tuberculous tracheobronchitis, J. Thoracic Surg., 1947, *16*, 512.

(6) Curreri, A. R., Gale, J. W., Dickie, J. A., and Longley, B. J.: Thoracoplasty and pulmonary resection in the treatment of tuberculous tracheobronchitis, Am. Rev. Tubere., 1948, *58*, 15.

(7) Overholt, R. H., Langer, L., Szypulski, J. T., and Wilson, N. J.: Pulmonary resection in the treatment of tuberculosis, J. Thoracic Surg., 1946, *15*, 384.

(8) McIndoe, R. B., Steele, J. D., Samson, P. C., Anderson, R. S., and Leslie, G. L.: Quoted in Sweany, H. C., and Behm, H.

(9) Sweany, H. C., and Behm, H.: Tuberculosis of trachea and major bronchi, Dis. of chest, 1948, *14*, 1.
(10) Jackson, C. L., and Huber, J. F.: Correlated applied anatomy of the bronchial tree with a system of nomenclature, Dis. of Chest, 1943, *9*, 1.
(11) Minkousky, A.: Quoted in Sweany, H. C., and Behm, H.
(12) Wilbur, G. H.: Quoted in Sweany, H. C., and Behm, H.
(13) Meissner, W. A.: Quoted in Sweany, H. C., and Behm, H.
(14) Samson, P. C.: Quoted in Curreri, A. R., Gale, J. W., Dickie, H. A., and Longley, B. J.
(15) Brewer, L. A., III, and Dolley, F. S.: Indications for pulmonary resection for tuberculosis both by lobectomy and pneumonectomy, Dis. of Chest, 1948, *14*, 491.
(16) Sweet, R. H.: Lobectomy and pneumonectomy in the treatment of pulmonary tuberculosis, J. Thoracic Surg., 1946, *15*, 373.
(17) Bailey, C. P.: Lung resection for pulmonary tuberculosis, J. Thoracic Surg., 1947, *16*, 328.
(18) Buckles, M. G.: Necessity of bronchoscope in modern chest work, Am. Rev. Tubere., 1937, *35*, 581.

THE USE OF GELATIN FOAM (GELFOAM) IN THORACOPLASTIES[1]

GUSTAF A. HEDBERG, WILLIAM D. SEYBOLD, AND O. THERON CLAGETT

(Received for publication September 7, 1949)

INTRODUCTION

The satisfactory collapse of the lung immediately after rib resection in thoracoplasty with the subsequent disappointing partial re-expansion is known to all. Because of this phenomenon, it has been necessary to sacrifice a considerable portion of the functioning pulmonary tissue to ensure closure of the apical cavities. Due to the fact that the subscapular reaction disappears before the ribs have regenerated sufficiently to produce a firm barrier to re-expansion, thoracoplasties limited to five ribs or less have not been practical except for favorably situated small cavitary lesions (figure 1). In the majority of thoracoplasties performed for apical cavities, therefore, at least seven ribs have been resected. This has resulted in sacrifice of functioning portions of the lungs and production of undue thoracic deformity.

In an attempt to avoid the sacrifice of functioning pulmonary tissue which accompanies classical thoracoplasties, many alternate procedures have been devised. Most of these operations have been discarded or their use limited because of complications. Apicolysis with plumbage, the procedure by which quantities of paraffin were inserted into an extrapleural space created surgically, has largely been abandoned because the paraffin tended to migrate or to be extruded from the space either through the chest wall or through pulmonary tissue. Use of extrapleural pneumothorax with subsequent maintenance of the collapse by mineral oil has been greatly limited by the tendency for the development of empyema with or without bronchopleural fistula, even many years after its introduction. The use of plastic (Lucite) balls has been attempted with the subsequent extrusion of this material. The introduction of any permanent foreign body into the tissues is contrary to surgical principles and has subsequent penalties. Limited thoracoplasties combined with apicolysis, as described by Semb, theoretically provided a solution, but too often the cavity remained open and resection of additional ribs was necessary for the control of the disease process.

In the search for an innocuous substance with which to fill the pleural space and stabilize the mediastinum following pneumonectomy, gelatin foam has been tried in experimental animals by Small, Clagett, and Grindlay. Its use for this purpose followed the demonstration by Jenkins and his co-workers and others that the substance was well tolerated by tissues, was without significant antigenic properties, and was an excellent hemostatic agent. When Small, Clagett, and Grindlay placed it in the pleural cavity in dogs and rats following pneumonectomy, it served as a satisfactory space-filler which prevented shift of the

[1] From the Nopeming Sanatorium, Nopeming, Minnesota, and the Division of Surgery, Mayo Clinic, Rochester, Minnesota.

mediastinum during the early postoperative period. There was no evidence of interference with the mechanisms which combat bacterial growth or effect repair. The material usually was completely absorbed in six to twelve weeks (figure 2).

Gelatin foam is a crisp, spongy mass which can be obtained in essentially unlimited quantities. It is inexpensive, easily handled, and light. One cubic centimeter weighs only 9 mg. and is capable of absorbing forty-five times its weight in blood or other fluid. Its satisfactory behavior in the pleural space in

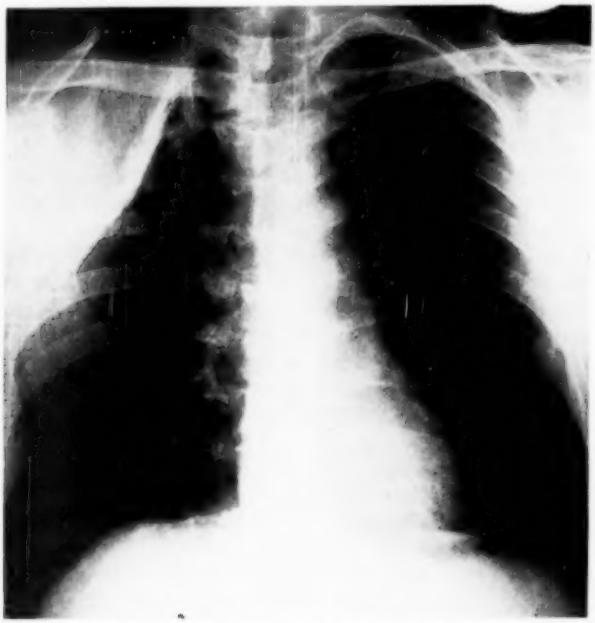


FIG. 1. A five-rib, two-stage thoracoplasty had been performed two and one-half years previously without the use of gelatin foam pack. Patient was a man 28 years of age.

these experiments led one of us (O. T. C.) to suggest and try it in the extrathoracic space in thoracoplasty. The purpose of this paper is to report on its use in this manner.

CLINICAL MATERIAL

The report is based on thoracoplasties performed in 11 patients at Mineral Springs Sanatorium, Cannon Falls, Minnesota, and in 29 patients (30 thoracoplasties, one was bilateral) at Nopeming Sanatorium, Nopeming, Minnesota. In all cases thoracoplasty was completed before July 1, 1948. Obviously it is too early to analyze the end results in these cases and, therefore, attention is

being focused on the mechanical results achieved and the complications encountered.

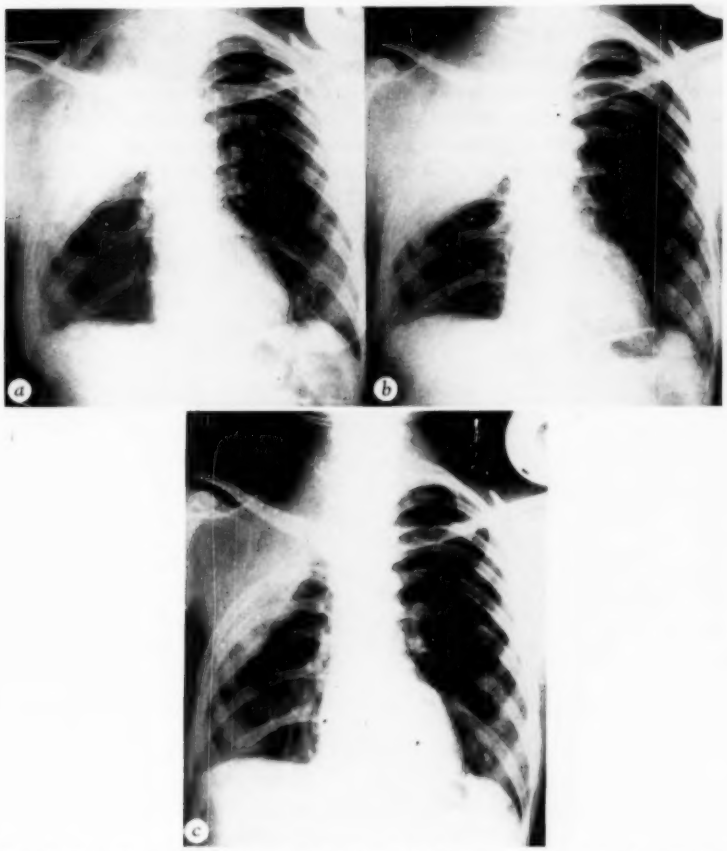


FIG. 2. Five-rib, one-stage thoracoplasty in which four gelatin-foam packs were used. (*a*) Six days after operation; (*b*) twenty-six days after operation; (*c*) seventy-nine days after operation. Patient was a woman 37 years of age.

Technique

It was found best to insert gelatin foam in its dry form after cutting or breaking pieces to fit the space to be packed. Care was used to insert the first piece well in front of the anterior portion of the perichondrium of the first and second costal cartilages. The pieces of gelatin foam were placed in the subscapular space in order to reconstruct the normal contour of the chest wall. Only the slightest

compression was used. In the first cases the material was soaked in saline solution and compressed before insertion. Too much packing was used and too much tissue collapsed. Occasionally the tissues were sprayed with penicillin before inserting the gelatin foam. The best results were obtained when five pads measuring 1.2 by 8.5 by 25.0 cm. were used after resection of five ribs for a man of average size, and somewhat less for a woman. When more than five ribs were resected at one stage, a proportionately greater amount was used. When gelatin foam was used in more than one stage of an operation in the same patient, the pack from the previous operation was removed and the space was repacked with fresh gelatin foam. The material when removed was found to be of the consistency of unorganized blood clot, reddish-brown in color, and bathed in serum-like fluid. Little or no tissue reaction was noted and no subscapular adhesions were encountered.

RESULTS

Cases at Mineral Springs Sanatorium

Gelatin foam was first used for packing in the course of thoracoplasty at Mineral Springs Sanatorium, and previous to July 1, 1948 it had been used in 11 cases there. Two patients had undergone resection of five ribs in one stage; 6, of seven ribs in two stages; and 3, of eight ribs in two stages. In the multiple-stage operations, gelatin foam inserted at the first stage was removed and the space repacked at the final stage. The mechanical results were excellent in 8, good in one, and fair in one. One patient died forty-eight hours after operation. This death followed a single-stage thoracoplasty in which seven ribs were resected. Paradoxic respiration seemed to be the underlying cause. In this case, only five pads of 1.2 by 8.5 by 25.0 cm. were used, a quantity which apparently was insufficient to stablize the chest wall. In the one case in which the mechanical result was classified as good the thoracoplasty was done over the site of an old empyema. A persistent postoperative sinus is healing slowly. Acid-fast bacilli have been recovered from the wound.

Cases at Nopeming Sanatorium

At Nopeming Sanatorium, gelatin foam was used for packing in three revision thoracoplasties. Closure of the cavities was accomplished in only one case. It was apparent that even when large quantities were used, the pack did not persist sufficiently long to accomplish the purpose. In one case, seven ribs were removed in a single stage after a preliminary anterolateral resection of the anterior ends of the upper three ribs. In 6 cases, the usual resection of seven ribs in multiple stages was employed. This consisted of a posterior stage on upper ribs. Three ribs and one-half of the fourth rib were removed. This was followed by an anterolateral stage with removal of the anterior segments of the next three ribs, the fifth, sixth, and seventh ribs. The final procedure was another posterior stage at which the posterior segments of the fifth, sixth, and seventh ribs were removed. In one case in which the seven-rib thoracoplasty was performed in multiple stages, gelatin foam was used in both posterior stages; in the remainder, it was

used only at the time of resection of the lower ribs. In one case, eight ribs were resected in four stages (figure 3) and in one, eleven ribs in four stages. In the four-stage procedure, gelatin foam was used in the two lower posterior stages. In the group of cases of thoracoplasties, with resection of five ribs, the first 7 patients underwent a preliminary anterolateral stage and the last 10 had their thoracoplasties done in a single stage. One patient in this group had bilateral thoracoplasty. In addition, one patient had six ribs removed in one stage. The transverse processes were not removed in any patient of this series. Postoperative thoracoplasty jackets were not used in any case. In an occasional case, sand bags were used for a few days after operation and a firm chest binder was applied for the first week.

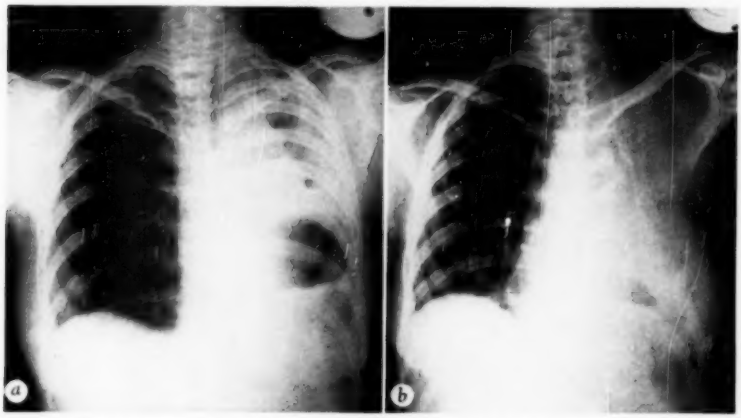


FIG. 3. Massive cavitation in the left lung and empyema in a patient 30 years of age. (*a*) Four-rib thoracoplasty completed; (*b*) three weeks after completion of an eight-rib, four-stage thoracoplasty. Gelatin foam was used at each posterior stage.

Little difficulty with paradoxic motion of the chest wall was experienced, in spite of the fact that in most of the patients there was no pleural thickening. A free pleural space was encountered in 6 cases.

The experience in the revision thoracoplasties has already been noted. The group of patients who underwent multiple-stage thoracoplasties is small. We have failed to demonstrate a persistent cavity in any of these patients who underwent multiple-stage operations and it is our impression that the gelatin-form pack has improved the degree of collapse. The elimination of the postoperative thoracoplasty jacket has made convalescence more comfortable.

Seventeen patients underwent five-rib thoracoplasties. Satisfactory collapse was obtained by 15. In 2, cavitation was persistent. In one of these, bilateral thoracoplasty had been done and more extensive rib resection was contraindicated because of the extent of the disease and bilateral cavitation. This patient had limited respiratory reserve and the postoperative course was marked by

dyspnea which required prolonged use of oxygen. In the other case, bilateral thoracoplasty was contemplated, but the appearance of dyspnea after unilateral

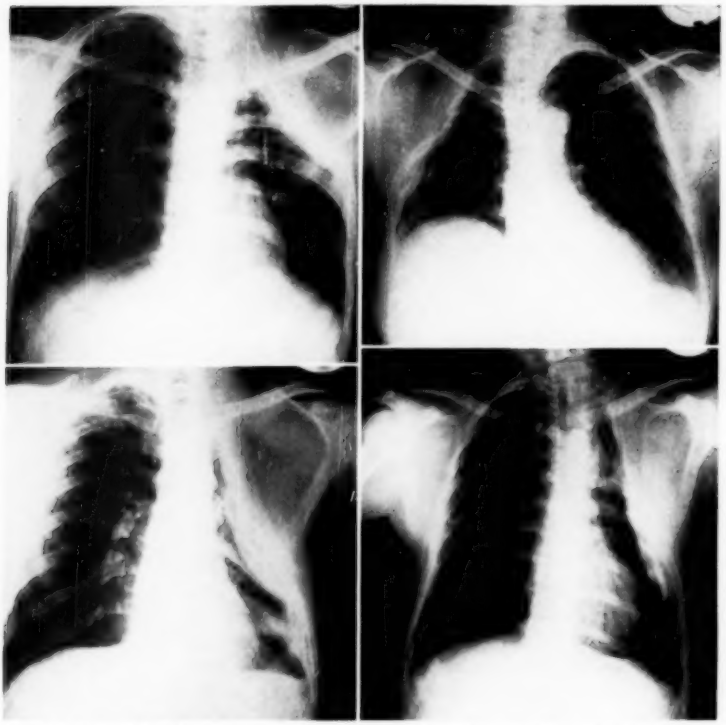


FIG. 4. (Upper left) Five-rib, one-stage thoracoplasty six months previously. Five packs of gelatin foam used. Patient was a man aged 22 years.

FIG. 5. (Upper right) Five-rib, one-stage thoracoplasty completed six months previously. Early collapse was adequate. The poor ultimate collapse is thought to be due to failure of the gelatin foam to persist until regeneration of the ribs had occurred. Patient was a woman 48 years old.

FIG. 6. (Lower left) Condition five months after seven-rib, one-stage thoracoplasty in which six packs of gelatin foam were used. Patient was a man 29 years of age.

FIG. 7. (Lower right) Condition five months after five-rib, one-stage thoracoplasty. Too much (six packs) of gelatin foam was used. Patient was a man 24 years of age.

thoracoplasty and the development of severe psychosis precluded any additional procedure. The patient required oxygen for eleven days after surgery.

The most satisfactory mechanical results were obtained in the younger group of patients, although cavity closure resulted in 15 cases in which five-rib thoracoplasties were performed in a single stage. Maximal collapse was obtained in

all patients who were less than 30 years of age (figure 4), while the final mechanical result was not as satisfactory in patients who were more than 45 years of age (figure 5). Apparently, periosteal regeneration is not as rapid in these patients and partial re-expansion occurs due to the absorption of the gelatin foam before the underlying thoracic cage has become rigid. In one patient, seven ribs were removed in one stage with no embarrassment of respiration (figure 6), and in another six ribs were removed. In 3 patients, overpacking was done. In one (the second patient operated on), seven pieces of gelatin foam which had previously been soaked in saline and compressed were placed in the wound. The wound opened on the fourteenth postoperative day and a portion of the liquified pack drained away. Micrococcus pyogenes var. albus (*Staphylococcus albus*) was cultured from the drainage material. In another in which seven pads were used for a five-rib thoracoplasty, a spread occurred in the base of the lung on the same side. This was the only instance of postoperative spread in the series. The third patient had no complications, but the final result of the removal of five ribs was equal to the usual result of a seven-rib thoracoplasty (figure 7).

Infection: Infection due to green-producing streptococci occurred in one surgical wound in which gelatin foam was used. Although the infection was overcome with penicillin, it must be borne in mind that gelatin foam plus serum makes a good culture medium. It might be wise to use penicillin routinely with the pack.

In the 6 patients in whom the pleural space was inadvertently opened and pneumothorax resulted, the presence of the pack did not affect the treatment of this complication. The underlying lung expanded satisfactorily in each case on aspiration of the air from the space. There were no deaths in this series of 29 cases.

COMMENT

In this study the impression was gained that the use of gelatin foam as a pack in conjunction with thoracoplasty has several advantages: (*1*) It increases the degree of collapse of the underlying lung and permits limited resection of ribs with the conservation of more functioning lung. (*2*) It decreases postoperative respiratory difficulties and eliminates the need for prolonged use of thoracoplasty binders. (*3*) It eventually disappears with little or no tissue reaction. Overpacking with this material should be avoided.

Among older patients, less favorable results are obtained than among younger patients because of slower regeneration of periosteum. It would be an advantage if a gelatin foam could be produced which would absorb more slowly than the present product. Gelatin foam has no bacteriostatic properties and may serve as a good culture medium. Perhaps penicillin and streptomycin should be used locally with it. As a result of the present experience with the gelatin-foam pack, it is believed that its continued trial can be recommended. It is also believed, however, that thoracoplasties of more than five ribs should not be attempted in a single stage when the pack is used unless there is evidence of little paradoxic motion at the time the ribs are removed.

REFERENCES

(1) Semb, C.: Thoracoplasty with extrafascial apicolysis, Acta chir. Scandinav., 1935, *76* (supp. 37), 1.

(2) Small, J. T., Clagett, O. T., and Grindlay, J. H.: Additional use of gelatin foam in thoracic surgery: Preliminary report, Proc. Staff Meet., Mayo Clin., 1947, *22*, 585.

(3) Jenkins, H. P., Senz, E. H., Owen, H. W., and Jampolis, R. W.: Present status of gelatin sponge for the control of hemorrhage; with experimental data on its use for wounds of the great vessels and the heart, J.A.M.A., 1946, *132*, 614.

STUDIES ON THE RESPIRATORY GAS EXCHANGE IN ONE HUNDRED ANTHRACITE COAL MINERS WITH PULMONARY COMPLAINTS[1,2]

HURLEY L. MOTLEY, LEONARD P. LANG, AND BURGESS GORDON

(Received for publication July 30, 1949)

INTRODUCTION

In a previous paper (1), measurements of pulmonary emphysema and ventilation volumes of 100 anthracite coal miners with respiratory complaints were reported. In the present study of a similar group of 100 cases, the physiological measurements have been obtained for appraising the impairment of respiratory gas exchange in the transfer of oxygen and carbon dioxide between alveoli and arterial blood. The data relating to the respiratory gas exchange are based on studies of arterial blood and expired air, and have been correlated with the degree of pulmonary emphysema. In this present group of 100 unselected anthracite miners, the incidence of emphysema as based on quantitative measurements of residual air was approximately the same as previously reported (1). In some instances data are used from the same case for both aspects of the physiological measurements of pulmonary function (pulmonary emphysema and ventilation volumes, and respiratory gas exchange data). As previously shown, emphysema accounts for only a part of the impairment; in fact, in a considerable number of these cases the degree of emphysema present is not significant (1). This report provides further information useful in the evaluation of the degree of pulmonary function impairment.

METHODS

Arterial blood samples were obtained from the brachial artery both at rest and immediately after exercise, an indwelling Cournand-type needle being used for the purpose. The blood was collected by the technique described by Riley (2), using heparin to prevent clotting and a small globule of mercury in the syringe to facilitate mixing. The blood sample was well shaken immediately after drawing in order to provide uniform mixing. The oxygen content and capacity and the carbon dioxide content were determined on the Van Slyke-Neill apparatus (3) using the Roughton (4) modification of the Sendroy method (5) for the oxygen capacity. Duplicate samples were run by two trained technicians using separate machines. Accurate checks were required on each blood sample. The per cent oxygen saturation was calculated by dividing the oxygen content by the oxygen capacity after suitable corrections had been made for the amount of oxygen dissolved in the plasma.

The arterial blood tension of oxygen and of carbon dioxide were obtained directly in mm. of mercury, using the method of Riley (2). All tension determinations were carried out immediately after the blood was drawn. Blood specimens for the tension measurements were collected simultaneously with the expired air samples, so that the blood sample ob-

[1] From the Cardio-Respiratory Laboratory, Barton Memorial Division of Jefferson Medical College Hospital, and the Department of Medicine of Jefferson Medical College, Philadelphia, Pa.

[2] Investigation aided by a research grant of the Anthracite Health and Welfare Fund of Jefferson Medical College Hospital.

tained would correspond to the R.Q. (respiratory quotient) which thus could be used in calculating the mean alveolar oxygen partial pressure (pO_2) by the indirect method (6), in which the arterial pCO_2 and alveolar pCO_2 are assumed to be the same. The indirect method for calculating the alveolar pO_2 was satisfactory since it provided consistent results in repeated determinations even after prolonged periods of time (several months or more). The Haldane method for calculating alveolar pO_2 was found unsatisfactory, as duplicate check values could not be obtained consistently even on the same patient during the same study period. The arterial blood pH was determined directly by a glass electrode to which the sample was transferred directly from the pipette, avoiding contact with air.

In calculating the oxygen gradients of pressure between the inspired air and the alveolus, correction was made for the difference in per cent of nitrogen in the inspired and expired air. The difference in oxygen partial pressure between the inspired air and the alveolus was referred to as the *aeration gradient*. The difference in oxygen partial pressure between the arterial blood and the alveolus was designated as *transfer gradient*, since it denotes the movement of oxygen from the gaseous state in the alveolus to either combination with hemoglobin or solution in the blood plasma.

Oxygen uptake and carbon dioxide output were calculated from the percentage of oxygen and carbon dioxide in the expired air as determined in a Scholander gas analyzer (7). The air breathed during the study period was supplied from a compressed air tank by a demand regulator[3]. The exact percentage of oxygen and carbon dioxide was determined on each tank full of compressed air. All expired air samples were collected over six minute intervals except when arterial tension measurements were made, in which case the expired air was collected for the ninety seconds simultaneously with the sampling of arterial blood from the brachial artery. All resting gas measurements were made in duplicate, and if the R.Q. was less than 0.7 for the resting sample or 0.6 for the exercise sample, the determination was repeated to test for air leaks. The minute ventilation was measured during the basal rest period and during one minute of step-up exercise. The exercise consisted of stepping up and down thirty times in one minute on a stool 20 centimeters high. The oxygen uptake and carbon dioxide output and minute ventilation were expressed at S.T.P. (standard temperature pressure). The average tidal air was determined by dividing the total minute ventilation volume by the respiratory rate obtained on a permanent kymograph tracing. All resting measurements were made with the patient in a fasting state, lying supine, and as flat as comfortable.

The effective tidal air was calculated from the arterial pCO_2 and the expired pCO_2, with corrections for the volume of dead space in the breathing valve[4] apparatus. The alveolar pCO_2 was assumed to be the same as the arterial pCO_2.

$$\text{Dead space air} = \text{tidal air} \times \frac{\text{alveolar } pCO_2 - \text{expired } pCO_2}{\text{alveolar } pCO_2} - \text{dead space in apparatus}$$

$$\text{Effective tidal air} = \text{tidal air} - \text{dead space air}$$

The duration of dyspnea (shortness of breath) as reported by the patient was timed following the step-up exercise test previously described.

MATERIAL FOR STUDIES

All of the patients were male anthracite coal miners with a long history of work in the mines, similar to the group previously reported (1). They were admitted in order of date of application; hence there was no selection by the laboratory staff except that cases with proved pulmonary tuberculosis, cardiac decompensation, malignancy of the lung, and occasionally other disabling medical

[3] Made by Scott Aviation Corporation.

[4] Made by Bishinger-Koehler Co., Pittsburgh, Pa.

conditions were omitted from this study. Dyspnea, cough, expectoration, weakness, weight loss, chest pain, and hemoptysis were the major complaints, with the greatest frequency of occurrence in the order listed (8, 9). There was no evidence of cardiac decompensation in any of the patients of this series at the time of the pulmonary function studies. There were 11 cases, however, with definite electrocardiographic evidence of myocardial damage other than a typical right heart strain pattern.

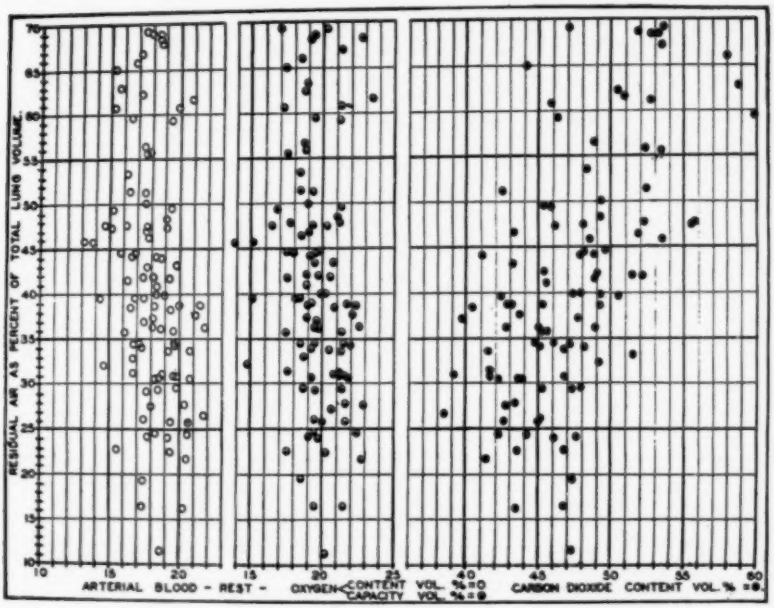

RESIDUAL AIR AS PERCENT OF TOTAL LUNG VOLUME
ARTERIAL BLOOD - REST -
OXYGEN-CONTENT
CAPACITY
VOL. % = O
VOL. % = O
CARBON DIOXIDE CONTENT VOL. % = O

FIG. 1. Relationship between arterial blood gases (oxygen content and capacity and carbon dioxide content in volumes per cent by Van Slyke method) and the degree of emphysema.

RESULTS

Respiratory Gas Exchange

The relationship between the arterial blood gases expressed in volumes per cent and the degree of emphysema is shown in figure 1. The average arterial blood oxygen content varied from 16 to 19 volumes per cent in the five groups regardless of the degree of emphysema (table 1). The relationship of arterial oxygen capacity in volumes per cent was somewhat similar to that of oxygen content (figure 1). It is of interest to note that most anthracosilicotic patients even with a far advanced degree of emphysema did not exhibit polycythemia or an increased hemoglobin. In contrast to oxygen content, the carbon dioxide

TABLE 1

Measurements on Ventilation, Emphysema, and the Respiratory Gas Exchange in One Hundred Anthracite Miners

	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V	
	Degree of Emphysema									
	None		Slight		Moderate		Advanced		Far Advanced	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
1. Number of cases	11		25		30		16		18	
2. Age in years	45	(30 to 57)	51	(30 to 71)	51	(40 to 60)	51	(39 to 63)	54	(38 to 67)
3. Inside mine, exposure, years	25	(4 to 40)	29	(10 to 47)	30	(15 to 42)	29	(15 to 45)	34	(14 to 56)
4. B.S.A. sq.M.	1.91	(1.74 to 2.08)	1.79	(1.60 to 2.12)	1.75	(1.23 to 2.08)	1.73	(1.58 to 1.90)	1.71	(1.50 to 1.98)
5. Maximal breathing capacity										
observed, L. per minute	111	(65 to 136)	83	(43 to 123)	63	(24 to 117)	43	(32 to 76)	26	(11 to 40)
percent of normal predicted value	92	(54 to 125)	75	(42 to 114)	62	(24 to 100)	41	(24 to 63)	27	(11 to 38)
6. Vital capacity										
observed, cc.	4,175	(3,600 to 5,300)	3,600	(2,500 to 4,400)	2,800	(1,850 to 4,350)	2,300	(1,485 to 4,060)	1,800	(1,310 to 2,400)
per cent of normal predicted value	100	(89 to 112)	89	(64 to 107)	71	(47 to 99)	53	(38 to 92)	44	(35 to 57)
7. Residual air										
observed, cc.	1,092	(698 to 1,510)	1,398	(1,064 to 2,187)	1,903	(1,223 to 3,020)	2,151	(1,294 to 3,540)	3,115	(1,700 to 4,860)
per cent of total lung volume	20.8	(11.8 to 24.8)	30.7	(25.8 to 34.8)	40.0	(35.6 to 44.9)	48.4	(45.6 to 53.5)	63.1	(55.2 to 69.4)
8. Arterial blood:										
A. CO_2 content, Volume per cent										
Rest	45.16	(41.37 to 47.63)	44.08	(38.49 to 51.40)	46.34	(39.75 to 52.20)	49.31	(42.55 to 55.74)	51.93	(44.10 to 60.13)
Exercise	41.79	(36.85 to 45.18)	42.26	(36.13 to 50.67)	43.53	(36.60 to 50.30)	47.48	(37.71 to 51.95)	49.54	(44.33 to 57.30)
B. O_2 content, Volume per cent										
Rest	18.64	(15.60 to 20.68)	18.76	(14.68 to 20.82)	18.20	(14.32 to 21.84)	16.67	(13.16 to 19.37)	17.40	(15.23 to 19.95)
Exercise	19.80	(15.55 to 22.17)	19.60	(15.5 to 21.68)	18.50	(13.92 to 21.36)	16.33	(13.42 to 19.25)	17.00	(14.08 to 20.27)
C. Hemoglobin, per cent saturation (O_2)										
Rest	92.8	(89.3 to 98.5)	93.6	(88.3 to 98.2)	93.3	(85.3 to 96.4)	91.0	(79.3 to 96.0)	90.5	(83.0 to 95.4)
Exercise	92.9	(88.6 to 97.7)	93.1	(88.0 to 97.8)	89.7	(79.8 to 95.5)	84.8	(76 to 93.3)	83.0	(72.0 to 93.0)
D. Mean resting tensions, mm. of mercury										
pCO_2	40	(36 to 45)	40	(24 to 50)	42	(27 to 52)	44	(20 to 55)	51	(37 to 62)
pO_2	79	(55 to 95)	78	(56 to 104)	76	(59 to 95)	71	(46 to 87)	66	(46 to 83)

E. pH₂										
Rest	7.48	(7.43 to 7.56)	7.48	(7.41 to 7.60)	7.47	(7.42 to 7.56)	7.47	(7.41 to 7.56)	7.44	(7.39 to 7.50)
Exercise	7.44	(7.41 to 7.51)	7.43	(7.37 to 7.47)	7.42	(7.36 to 7.48)	7.41	(7.34 to 7.48)	7.40	(7.29 to 7.48)
9. Resting alveolar pO₂, mm. of mercury	105	(95 to 108)	101	(84 to 128)	98	(82 to 120)	97	(82 to 129)	87	(72 to 101)
10. Resting oxygen gradients, mm. of mercury										
Aeration	45	(35 to 54)	49	(23 to 68)	51	(30 to 66)	53	(30 to 70)	63	(49 to 77)
Transfer	26	(14 to 40)	23	(4 to 41)	22	(7 to 48)	26	(10 to 42)	21	(8 to 39)
11. Air breathed										
A. Ventilation L. per min., per Sq.M. B.S.A.										
Rest	4.6	(3.7 to 7.7)	5.0	(3.1 to 8.6)	5.1	(3.5 to 7.6)	5.2	(3.0 to 8.4)	5.1	(3.7 to 6.1)
Exercise	12.1	(8.0 to 17.5)	12.8	(8.3 to 20.6)	12.3	(9.1 to 20.9)	11.0	(7.2 to 14.1)	10.5	(8.4 to 13.4)
B. Per cent CO_2 added										
Rest	2.78	(2.41 to 3.15)	2.42	(1.78 to 3.36)	2.35	(1.86 to 3.19)	2.46	(1.72 to 3.77)	2.43	(1.97 to 3.31)
Exercise	3.19	(2.98 to 3.57)	2.73	(1.96 to 3.80)	2.75	(1.94 to 3.21)	2.65	(2.22 to 3.07)	2.48	(2.17 to 3.06)
C. Per cent O_2 removed										
Rest	3.22	(2.47 to 3.90)	2.94	(1.68 to 4.06)	2.86	(2.20 to 3.93)	2.98	(1.72 to 4.70)	2.98	(2.35 to 4.26)
Exercise	4.48	(3.63 to 5.99)	3.96	(2.64 to 5.49)	3.77	(1.82 to 6.16)	3.65	(2.52 to 4.50)	3.12	(2.37 to 3.99)
D. Oxygen consumption, cc. per minute, per Sq.M. B.S.A.										
Rest	148	(126 to 184)	147	(123 to 167)	146	(114 to 191)	155	(129 to 188)	152	(118 to 177)
Exercise	542	(423 to 778)	507	(337 to 820)	464	(264 to 828)	402	(276 to 706)	328	(225 to 537)
E. Resting tidal air observed, cc.	490	(417 to 900)	470	(291 to 680)	469	(285 to 723)	510	(350 to 808)	420	(309 to 608)
Per cent effective	64	(57 to 73)	59	(48 to 78)	58	(46 to 72)	58	(45 to 68)	52	(44 to 63)
12. Dyspnea after exercise, sec.	104	(64 to 145)	146	(54 to 299)	142	(65 to 302)	186	(60 to 490)	225	(75 to 340)

content in volumes per cent did show a correlation with the degree of pulmonary emphysema (figure 1 and table 1). In patients in whom the degree of pulmonary emphysema was insignificant, groups I and II (table 1; residual air 35 per cent or less of total lung volume), the arterial carbon dioxide content varied from 38 to 51 volumes per cent at rest and 36 to 51 volumes per cent during exercise. In patients with a significant degree of pulmonary emphysema, the carbon dioxide content in general rose as the degree of emphysema increased, reaching values as high as 55 to 60 volumes per cent in some cases with a far advanced

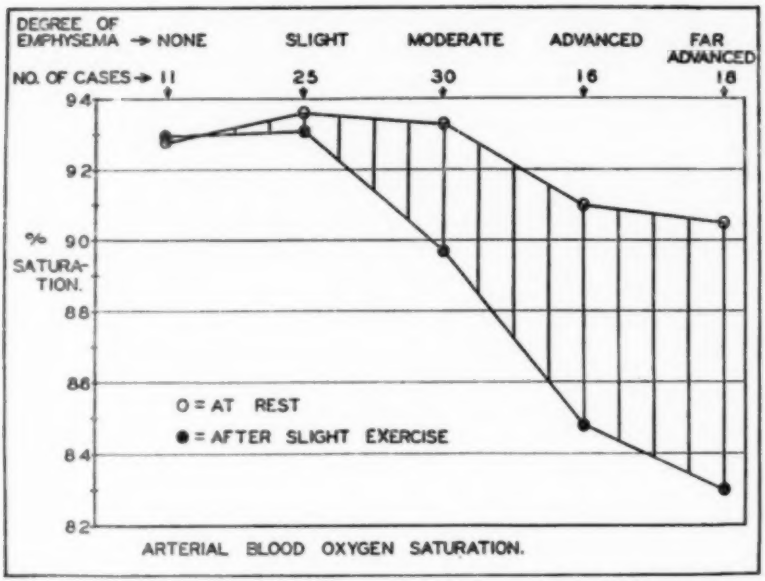


FIG. 2. A graphic representation of the correlation between arterial blood oxygen saturation and the degree of emphysema both at rest and immediately after exercise. Note that the arterial blood oxygen saturation decreases progressively after slight exercise in patients with a greater degree of emphysema, whereas the resting values show only slight changes. Normal arterial oxygen saturation 96 per cent or more.

degree of pulmonary emphysema (figure 1). Furthermore during exercise the decrease in carbon dioxide content was less in patients with extensive emphysema (table 1).

The arterial blood oxygen saturation, both at rest and immediately after exercise, was correlated with the degree of pulmonary emphysema (figure 2 and table 1). Most of the patients at rest had an average arterial oxygen saturation varying from 90 to 94 per cent regardless of the degree of emphysema, there being only a slight decrease in those with a far advanced degree of emphysema. However, the exercise arterial oxygen saturation data were strikingly different

from the resting values, and were correlated with the degree of pulmonary emphysema. In the miners with a significant degree of emphysema, the more marked the emphysema, the greater the decrease in oxygen saturation with exercise (figure 2). The exercise test produces a short period of acute hypoxia in most advanced emphysematous patients. Acute hypoxia even of short duration produces increased pulmonary artery pressure, decreased cardiac output, and increased pulmonary vascular resistance (10). A decrease in arterial oxygen saturation with exercise is highly significant in evaluating disability because the factors enumerated limit the magnitude of the increase in pulmonary blood flow

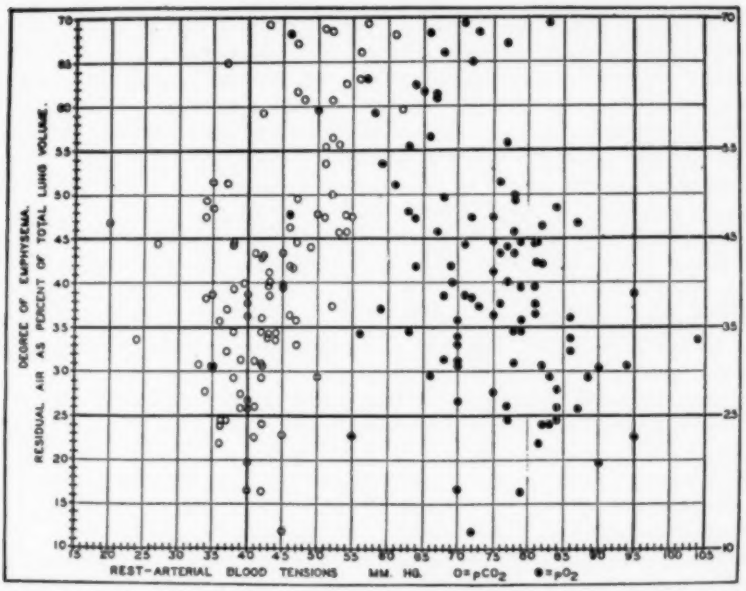


FIG. 3. Relationship between arterial blood tensions (direct Riley method) and the degree of emphysema. Normal arterial pO_2, 95 mm. of mercury and pCO_2, 40 mm. of mercury.

during exercise and work. In some individuals the arterial oxygen saturation may be essentially normal at rest, but decreases 10 to 15 per cent, or even more, with slight exercise.

The correlations between arterial blood tensions (pCO_2 and pO_2) and the degree of emphysema are shown in figure 3. In general the arterial pCO_2 value increased progressively in patients with a moderate to far advanced degree of pulmonary emphysema. In 2 patients with a far advanced degree of pulmonary emphysema the resting arterial pCO_2 was above 60 mm. of mercury. Hyperventilation of a sufficient magnitude to lower the arterial pCO_2 below 35 mm. of mercury was observed in several cases. In most instances, if the residual air

was less than 35 per cent of the total lung volume, the arterial pCO_2 was between 35 and 45 mm. of mercury. Although the arterial pCO_2 was elevated with emphysema as was the arterial CO_2 content, the arterial blood pH indicated that the patient was acclimated to the higher pCO_2 value (table 1). The resting pH value varied from 7.39 to 7.60 depending on the amount of apprehension and hyperventilation present. The average pH values showed small changes in the various groups (table 1). It has been found that breathing through a mouthpiece alone will increase the average arterial pH from .02 to .03 pH units.

Although there was a correlation between the arterial pCO_2 and the degree of pulmonary emphysema, there was very little variation in the arterial pO_2 with the degree of emphysema (figure 3). The arterial mean oxygen partial pressure (pO_2) was subnormal in all groups at rest (figure 3 and table 1) with a slightly greater decrease in arterial pO_2 in patients with advanced and far advanced degrees of emphysema. In a few patients with no pulmonary emphysema, the pO_2 was decreased markedly (in one patient as low as 55 mm. of mercury). In general, most of the pO_2 values lie between 55 and 85 mm. of mercury. In only 6 instances was the arterial pO_2 within normal limits and in one of these there was pronounced hyperventilation (figure 3). The progressive increase in arterial pCO_2 correlated with the degree of emphysema indicated the inability of many patients with an increased volume of residual air to blow off carbon dioxide adequately by hyperventilation. An increase in the arterial pO_2 and a decrease in the arterial pCO_2 with hyperventilation are significant findings with respect to disability evaluation, especially as compared to persistent low arterial pO_2 and high pCO_2 even in the presence of extensive hyperventilation.

The alveolar pO_2 values revealed a progressive decrease in the five groups correlated with the degree of emphysema (table 1). Thus, as the degree of emphysema increased, the alveolar pO_2 decreased. Although the alveolar pO_2 was calculated by the indirect method, repeated determinations on a number of anthracosilicotic cases after different time intervals demonstrated the reproducibility of the values.

The oxygen pressure gradients between the outside air and the arterial blood are shown in figure 4 and table 1. Aeration gradient was used to designate the difference of pressure in mm. of mercury between the effective inspired pO_2 and the mean alveolar pO_2. There was a correlation of the aeration gradient with the degree of emphysema (figure 4 and table 1) in which, in general, as the degree of emphysema increased the aeration gradient increased. In some patients with a far advanced degree of emphysema this value rose above 75 mm. of mercury (normal 50). In other cases the aeration gradient was below the normal value of 50 mm. of mercury (figure 4), including 9 patients with an advanced degree of emphysema. This would indicate that some miners were able to compensate for the increased volume of residual air more efficiently than others, and that they did so by hyperventilation which lowered the aeration gradient and increased the alveolar pO_2. In general, however, the greater the degree of pulmonary emphysema the larger the aeration gradient (figure 4).

Transfer gradient was used to designate the difference between the mean

alveolar pO_2 and the arterial pO_2 in mm. of mercury. Transfer thus indicates the movement of oxygen from a gaseous state across the pulmonary membrane to combine with hemoglobin or go in solution in the blood plasma. As shown in figure 4 and table 1, the transfer gradient was increased in all groups and there was no correlation with the degree of emphysema. In general, the transfer gradient was greater (22 mm. of mercury) for patients with no increased residual air than in patients with a far advanced degree of emphysema (19 mm. of mercury) (table 1). In one patient the transfer gradient was 40 mm. of mercury and

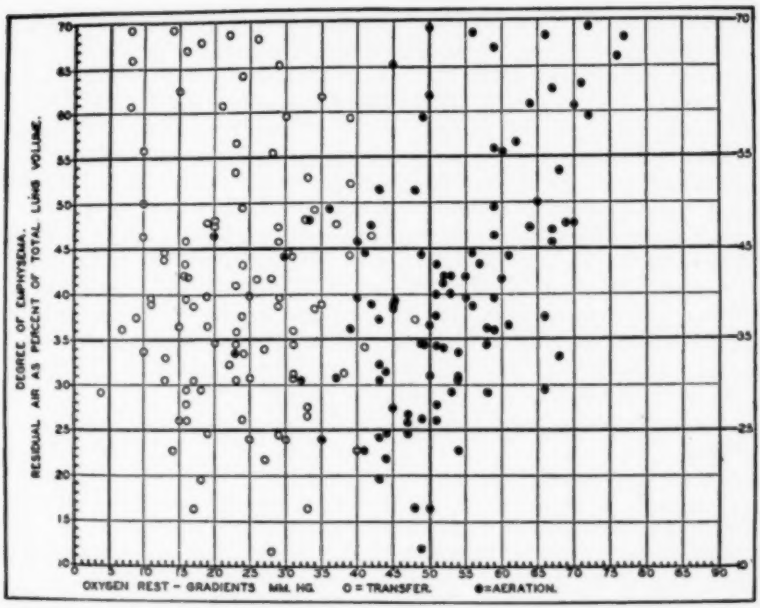


FIG. 4. Relationship between resting mean oxygen gradients of pressure and degree of emphysema. Transfer gradient refers to the mean oxygen pressure difference between alveoli and arterial blood (normal, 5 mm of mercury). Aeration gradient refers to the mean oxygen pressure difference between the inspired air and alveoli (normal, 50 mm of mercury).

the residual air was 22.5 per cent of the total lung volume (figure 4), while in 6 with an advanced degree of pulmonary emphysema the transfer gradient was within normal limits, indicating that most of the disability in these patients resulted from the increased residual air and the decreased ventilation. The increase in the transfer gradient may come from impaired diffusion, the result of an increased resistance at the pulmonary membrane to the movement of oxygen molecules from the alveolus to the blood, or from a distribution difficulty resulting from unequal alveolar aeration and perfusion.

The evidence from all data obtained in this study indicated that the difficulty

is almost entirely one of distribution. Studies carried out on more than 50 patients, using high and low levels of oxygenation, indicated that the increased gradient was related to unequal alveolar aeration and perfusion, and that oxygen moved across the pulmonary membrane in proportion to the partial pressures in the various alveoli.

Expired air was analyzed for the amount of oxygen removed and the amount of carbon dioxide added, both of these values being expressed as per cent. Thus 4.5 per cent oxygen removed from the air breathed indicates 45 cc. of oxygen removed from each liter of air breathed, and similarly 3.6 per cent of carbon dioxide added indicates 36 cc. of carbon dioxide added to each liter of air breathed. The values of oxygen uptake and carbon dioxide output pertain to ventilation efficiency and indicate in general the relationship between perfusion and ventilation in the lung. From the data which are given in table 1, the R.Q. can be determined and the oxygen consumption and the carbon dioxide output expressed and described as respiratory equivalents. The data presented in figure 5 show a lack of correlation between the degree of emphysema and the percentages of oxygen removed and carbon dioxide added to the air breathed. In most instances the per cent of oxygen removed and the per cent of carbon dioxide added were below the normal values (oxygen 4 to 5 per cent and carbon dioxide 3.2 to 4 per cent) (figure 5). The average data by groups (table 1) revealed little difference in the per cent of oxygen removal from the air breathed at rest with or without emphysema. A small progressive decrease in oxygen removal during exercise was correlated with the presence of emphysema (average 3.12 per cent in the far advanced cases of emphysema as compared to 4.48 per cent in those with no emphysema) (table 1). Individual variations were quite wide and unpredictable.

In general, changes in carbon dioxide addition to the air breathed varied similarly to oxygen removal both during rest and exercise (figure 5 and table 1). There was no demonstrable evidence that the carbon dioxide values were more significant than the oxygen values either at rest or during exercise. In some instances the low values for oxygen removal resulted partially from the marked hyperventilation of apprehension, but this alone does not account for the large decrease in carbon dioxide output associated with diminished oxygen uptake of similar magnitude.

The average oxygen consumption was approximately the same for each of the five groups at rest (table 1) varying from 147 to 155 cc. per minute per Sq.M., B.S.A. (square meter, body surface area) but during exercise there was a progressive average fall in oxygen consumption correlated with the extent of emphysema. In all groups, however, there were wide individual variations present. The decrease in the total oxygen consumption in the emphysematous patients during exercise was primarily related to the decreased minute ventilation and oxygen uptake from the inspired air (table 1). Apprehension at the time of study probably accounted for the somewhat elevated oxygen consumption rate. There was no evidence of hyperthyroidism in any of the patients.

As single measurements, the information pertaining to the per cent of oxygen

removed and the per cent of carbon dioxide added (even when expressed as respiratory equivalents) were of limited value in evaluating pulmonary function in anthracosilicosis. The reason for this is the fact that the ventilation efficiency was reduced in most of the cases and the reduction was just as marked even in patients with no pulmonary emphysema as in those with emphysema of a far advanced degree.

The average minute ventilation at rest varied from 4.6 to 5.2 liters per Sq.M., B.S.A. for all groups. There was no correlation with the degree of emphysema.

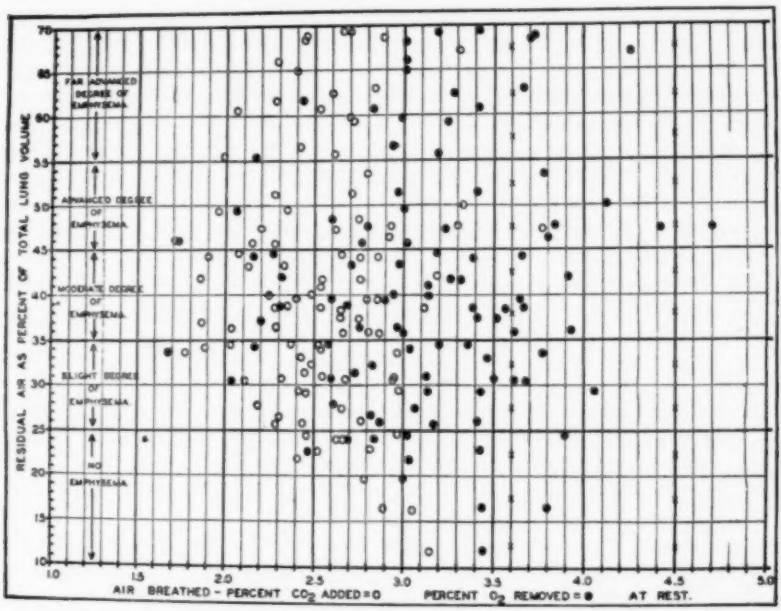

RESIDUAL AIR AS PERCENT OF TOTAL LUNG VOLUME
FAR ADVANCED DEGREE OF EMPHYSEMA
ADVANCED DEGREE OF EMPHYSEMA
MODERATE DEGREE OF EMPHYSEMA
SLIGHT DEGREE OF EMPHYSEMA
NO EMPHYSEMA
AIR BREATHED - PERCENT CO_2 ADDED = 0 PERCENT O_2 REMOVED = 0 AT REST.

FIG. 5. Relationship between the ventilation efficiency of the lung (as measured by the per cent of oxygen removed, and the per cent of carbon dioxide added, to the air breathed) and the degree of pulmonary emphysema. Note the lack of correlation between the degree of emphysema and oxygen removal and carbon dioxide addition. Normal CO_2 addition 3.2 to 4.0 per cent and O_2 uptake 4.0 to 5.0 per cent. During exercise these values are increased.

The average exercise minute ventilation was 12.1 to 12.8 liters per Sq.M., B.S.A. for groups I and II and 12.3 for group III, but in the miners with an advanced or far advanced degree of emphysema (groups IV and V) the average was 11.0 and 10.5, respectively (table 1). It may also be seen from table 1 that patients with an advanced or far advanced degree of emphysema did not show the wide individual ranges of ventilation with the high volumes during exercise seen in the other groups. Extensive emphysema definitely hampers compensatory hyperventilation.

When the tidal volume at rest was calculated by dividing the total minute ventilation by the respiratory rate, the average volume for tidal air was found to vary from 400 to 500 cc. in the five groups (table 1). The per cent of tidal air which was effective in alveolar aeration was calculated from the expired pCO_2 and the arterial pCO_2 with corrections for the dead space in the apparatus as previously described. The effective resting tidal air varied from 64 per cent in group I to 52 per cent in group V. There was a fair correlation of the effective tidal air with the degree of pulmonary emphysema throughout the five groups (table 1).

The relationship between the duration of dyspnea after one minute of step-up exercise and the degree of emphysema is shown in table 1. The average dyspnea time increased progressively in the five groups and was greater the more advanced the degree of emphysema. Wide individual variations were noted. In most instances, if the residual air was 50 per cent or more of the total lung volume, the duration of dyspnea was definitely prolonged. In some miners, however, the dyspnea was prolonged without a significant increase in residual air. It would appear from the data presented that dyspnea as a single measurement was of limited value in estimating the degree of pulmonary function impairment in anthracosilicosis unless the duration was markedly extended (three minutes or more). Since this measurement is a subjective one, individual variations could be expected to be more pronounced and the findings less reliable.

Case Studies

The studies including complaints, pulmonary function data, physiological evaluation, and chest roentgenograms are presented for 6 representative cases with varying degrees of pulmonary function impairment. Impairment was graded on the basis of the ability of the lungs to supply oxygen to the blood both while the subjects were at rest and during exercise. Although wide individual variations were found, the features presented by these measurements in any given case must be consistent with known physiological principles. For example, if the aeration gradient were markedly elevated (70 mm. of mercury) and yet no increase in the residual air per cent of total lung volume were noted, a contraindication would be evident and errors indicated.

The inspiratory and expiratory roentgenograms of the 6 cases reported were helpful in evaluating the degree of emphysema, but provided little information on the extent of the respiratory gas exchange involvement. The representative cases illustrate that the major factors producing pulmonary function impairment may act singly or in combination and with wide individual variations.

The studies on respiratory gas exchange revealed a lack of correlation between the extent of the function impairment and the roentgenographic findings, similar to observations and measurements previously reported on the correlation between the roentgenographic picture and the degree of pulmonary emphysema (1).

Case 1. P. Y. (figure 6) was a 39-year-old man who had been an anthracite miner for twenty-eight years. He experienced the onset of symptoms one year ago, consisting of pain in the upper anterior chest. He was advised by a local doctor to get out of the mines because of changes noted on the chest roentgenograms. No dyspnea, cough, or expectoration were present. The roentgenographic diagnosis was borderline-stage pneumoconiosis.

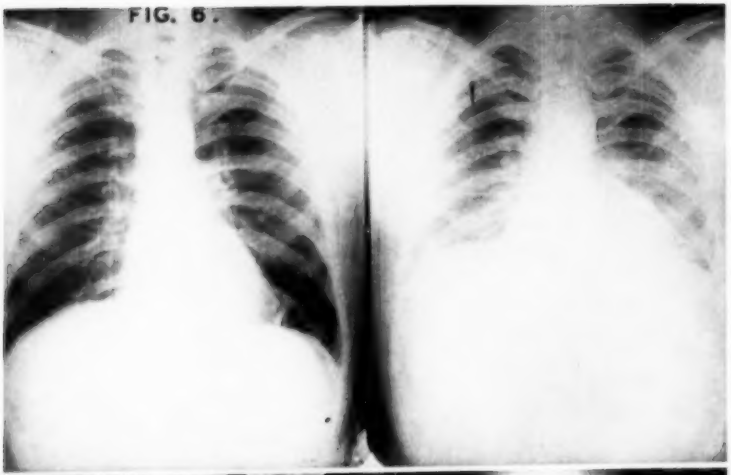
Pulmonary Function Studies	Observed	Predicted*
Maximal breathing capacity, L. per min.	106	(127)
Vital capacity, cc.	3,650	(4,110)
Residual air, cc.	1,065	(1,330)
Residual air per cent of total lung volume	22.6	(24.5)
Ventilation, L. per min. per Sq.M., B.S.A.		
Rest	5.5	(3.0 to 4.0)
Exercise	11.7	(8.0 to 11.0)
Ventilation efficiency, O_2 uptake		
Rest, amount of O_2 removed from air breathed	2.47	(4.5 per cent)
Exercise, amount of O_2 removed from air breathed	5.28	(5.5 per cent)
Arterial blood oxygen saturation		
Rest	97.5	(96+ per cent)
Exercise	97.7	(96+ per cent)
Arterial pCO_2, rest, mm. of mercury	41	(40)
Arterial pO_2, rest, mm. of mercury	95	(95)
Alveolar pO_2, rest, mm. of mercury	109	(100)
Mean gradient, *aeration*, pO_2, inspired air to alveolus, rest, mm. of mercury	41	(50)
Mean gradient, *transfer*, pO_2, alveolus to arterial blood, rest, mm. of mercury	14	(5)
Duration of dyspnea after exercise, sec.	111	(60)

* Predicted values for lung volumes based on the studies of Cournand and Richards and their coworkers (11, 12).

Evaluation: The patient had a very slight degree of pulmonary functional impairment due to a small decrease in ventilation (maximal breathing capacity and vital capacity). A slight increase in the transfer gradient resulted from hyperventilation which had elevated the alveolar pO_2.

Case 2. P. Yd. (figure 7) was a 45-year-old man who had been an anthracite miner for twenty-eight years. He first experienced symptoms four years ago with the onset of dyspnea during exercise and an occasional fainting episode. A slight cough and expectoration with a twenty pound weight loss had occurred during the past four years. He stopped working two years ago because of dyspnea which was severe with certain changes in weather. A roentgenographic diagnosis of second stage pneumoconiosis was made.

Pulmonary Function Studies	Observed	Predicted
Maximal breathing capacity, L. per min.	70	(116)
Vital capacity, cc.	2,960	(4,560)
Residual air, cc.	1,885	(1,480)
Residual air per cent of total lung volume	38.9	(24.5)
Ventilation, L. per min. per Sq.M., B.S.A.		
Rest	5.2	(3.0 to 4.0)
Exercise	14.4	(8.0 to 11.0)
Ventilation efficiency, O_2 uptake		
Rest, amount of O_2 removed from air breathed	2.73	(4.5 per cent)
Exercise, amount of O_2 removed from air breathed	4.37	(5.5 per cent)



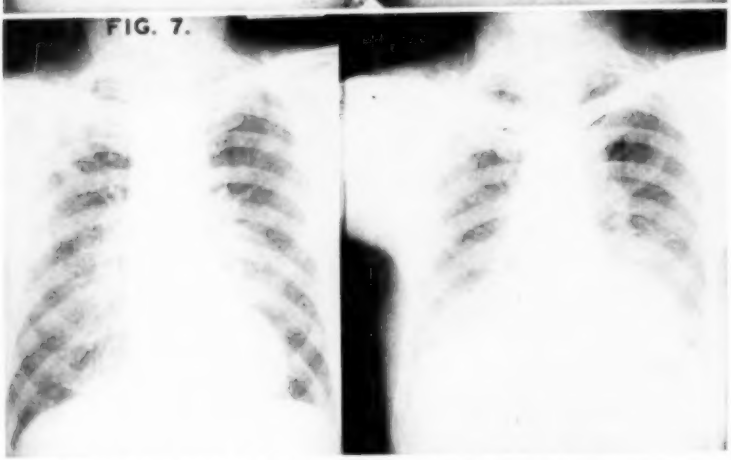


FIG. 6 (Top) Case 1, P. Y. Inspiratory and expiratory roentgenograms
FIG. 7 (Bottom) Case 2, P. Yd. Inspiratory and expiratory roentgenograms

Pulmonary Function Studies *(cont.)*	Observed	Predicted
Arterial blood oxygen saturation		
Rest	96.0	(96+ per cent)
Exercise	93.7	(96+ per cent)
Arterial pCO_2, rest, mm. of mercury	35	(40)
Arterial pO_2, rest, mm. of mercury	95	(95)
Alveolar pO_2, rest, mm. of mercury	106	(100)
Mean gradient, *aeration*, pO_2, inspired air to alveolus, rest, mm. of mercury	45	(50)
Mean gradient, *transfer*, pO_2, alveolus to arterial blood, rest, mm. of mercury	11	(5)
Duration of dyspnea after exercise, sec.	85	(60)

Evaluation: A slight to moderate degree of impairment of pulmonary function was present due to: decrease in ventilation, increase in residual air, decreased oxygen uptake from the air breathed, and a slight decrease in arterial oxygen saturation after exercise. Hyperventilation compensated well in this case, elevating the arterial pO_2 and decreasing the arterial pCO_2 and aeration gradient.

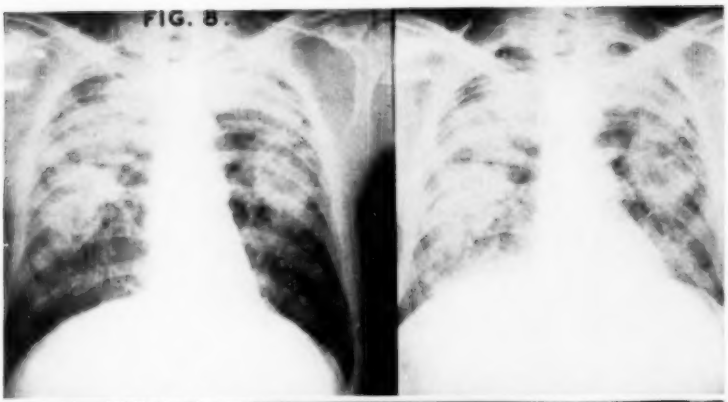
Case 3. A. M. (figure 8) was a 42-year-old man who had been an anthracite miner for twenty-three years. The onset of his symptoms occurred seven years ago when dyspnea of effort with gradual increasing severity appeared, but he was able to work until fifteen months ago. He had a slight cough and weakness but no weight loss or expectoration. A roentgenographic diagnosis of third stage pneumoconiosis was made.

Pulmonary Function Studies	Observed	Predicted
Maximal breathing capacity, L. per min.	69	(125)
Vital capacity, cc.	3,320	(4,390)
Residual air, cc.	1,041	(1,420)
Residual air per cent of total lung volume	23.9	(24.5)
Ventilation, L. per min. per Sq.M., B.S.A.		
Rest	5.0	(3.0 to 4.0)
Exercise	14.7	(8.0 to 11.0)
Ventilation efficienty, O_2 uptake		
Rest, amount of O_2 removed from air breathed	3.1	(4.5 per cent)
Exercise, amount of O_2 removed from air breathed	4.5	(5.5 per cent)
Arterial blood oxygen saturation		
Rest	87	(96+ per cent)
Exercise	88	(96+ per cent)
Arterial pCO_2, rest, mm. of mercury	39	(40)
Arterial pO_2, rest, mm. of mercury	74	(95)
Alveolar pO_2, rest, mm. of mercury	107	(100)
Mean gradient, *aeration*, pO_2, inspired air to alveolus, rest, mm. of mercury	45	(50)
Mean gradient, *transfer*, pO_2, alveolus to arterial blood, rest, mm. of mercury	33	(5)
Duration of dyspnea after exercise, sec.	144	(60)

Evaluation: There was present a moderate degree of pulmonary function impairment due to: the decrease in ventilation, the marked drop in arterial oxygen saturation and arterial pO_2, the large transfer gradient, and moderate prolongation of dyspnea after exercise. In spite of the more marked roentgenographic changes, the pulmonary function was less impaired in this case than in case 4 and much less than in case 5 (figures 9 and 10).

Case 4. A. Mo. (figure 9) was a 64-year-old man who had been an anthracite miner for twenty-eight years. The onset of his symptoms occurred eleven years ago with dyspnea, which increased progressively in severity. He stopped work one year ago because of dyspnea. He had moderate cough and expectoration, marked weakness, right lower chest pain, and had experienced a forty pound weight loss in the past eleven years. The roentgenographic diagnosis was second-stage pneumoconiosis.

Pulmonary Function Studies	Observed	Predicted
Maximal breathing capacity, L. per min.	78	(87)
Vital capacity, cc.	4,050	(3,900)
Residual air, cc.	2,090	(1,660)
Residual air per cent of total lung volume	34.0	(30.0)



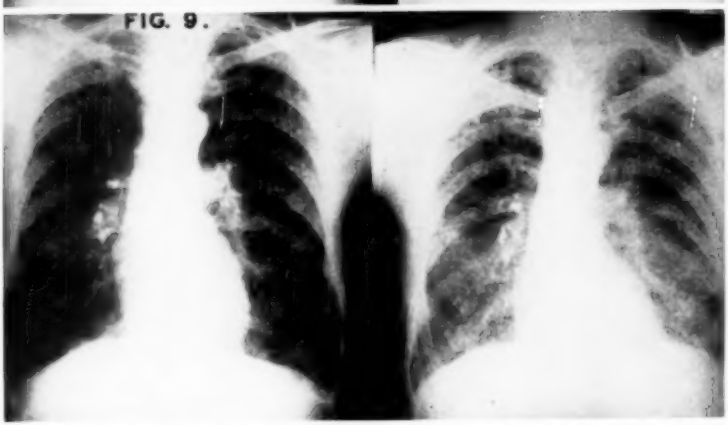


FIG. 8 (Top) Case 3, A. M. Inspiratory and expiratory roentgenograms.
FIG. 9 (Bottom) Case 4, A. Mo. Inspiratory and expiratory roentgenograms

Pulmonary Function Studies *(cont.)*	Observed	Predicted
Ventilation, L. per min. per Sq.M., B.S.A.		
Rest	4.5	(3.0 to 4.0)
Exercise	10.7	(8.0 to 11.0)
Ventilation efficiency, O_2 uptake		
Rest, amount of O_2 removed from air breathed	3.04	(4.5 per cent)
Exercise, amount of O_2 removed from air breathed	3.85	(5.5 per cent)
Arterial blood oxygen saturation		
Rest	91.2	(96+ per cent)
Exercise	94.0	(96+ per cent)
Arterial pCO_2, rest, mm. of mercury	43	(40)
Arterial pO_2, rest, mm. of mercury	70	(95)
Alveolar pO_2, rest, mm. of mercury	98	(100)
Mean gradient, *aeration*, pO_2, inspired air to alveolus, rest, mm. of mercury	52	(50)

Pulmonary Function Studies *(cont.)*	Observed	Predicted
Mean gradient, *transfer*, pO_2, alveolus to arterial blood, rest, mm. of mercury	26	(5)
Duration of dyspnea after exercise, sec.	299	(60)

Evaluation: The patient had a moderate degree of pulmonary function impairment due to: increased residual air, decreased oxygen uptake from inspired air, decreased arterial oxygen saturation at rest, decreased arterial pO_2, increased transfer gradient, and marked prolongation of dyspnea after exercise. The prolonged dyspnea suggests involvement of the pulmonary circulation with increased pulmonary vascular resistance during exercise as the major factor of impairment. Studies on pulmonary vascular resistance may reveal a greater degree of pulmonary function impairment than that recorded above.

Case 5. J. N. (figure 10) was a 39-year-old man who had been an anthracite miner for 23 years. The onset of his symptoms occurred five years ago with dyspnea which increased progressively in severity so that he stopped work two years ago. He has had a moderate degree of cough and expectoration and a twenty-five pound weight loss during the past year. The roentgenographic diagnosis was third-stage pneumonconiosis.

Pulmonary Function Studies	Observed	Predicted
Maximal breathing capacity, L. per min.	42	(123)
Vital capacity, cc.	2,480	(4,440)
Residual air, cc.	2,120	(1,420)
Residual air per cent of total lung volume	46.1	(24.5)
Ventilation, L. per min. per Sq.M., B.S.A.		
Rest	4.7	(3.0 to 4.0)
Exercise	9.3	(8.0 to 11.0)
Ventilation efficiency, O_2 uptake		
Rest, amount of O_2 removed from air breathed	2.73	(4.5 per cent)
Exercise, amount of O_2 removed from air breathed	3.06	(5.5 per cent)
Arterial blood oxygen saturation		
Rest	90.0	(96+ per cent)
Exercise	81.2	(96+ per cent)
Arterial pCO_2, rest, mm. of mercury	44	(40)
Arterial pO_2, rest, mm. of mercury	60	(95)
Alveolar pO_2, rest, mm. of mercury	92	(100)
Mean gradient, *aeration*, pO_2, inspired air to alveolus, rest, mm. of mercury	56	(50)
Mean gradient, *transfer*, pO_2, alveolus to arterial blood, rest, mm. of mercury	32	(5)
Duration of dyspnea after exercise, sec.	347	(60)

Evaluation: The patient had an advanced degree of pulmonary function impairment due to: decreased ventilation, increased residual air which produced an advanced degree of emphysema, decreased oxygen uptake, arterial oxygen unsaturation of a profound degree during exercise, decreased arterial pO_2, increased transfer gradient, and marked prolongation of dyspnea after exercise.

Case 6. J. C. (figure 11) was a 54-year-old man who had been an anthracite miner for thirty-five years. The onset of his symptoms occurred eight years ago with dyspnea which has progressively increased so that at present he is unable to work. He has had frequent disabling colds during all seasons. The dyspnea has been severe on slight effort and he has had a

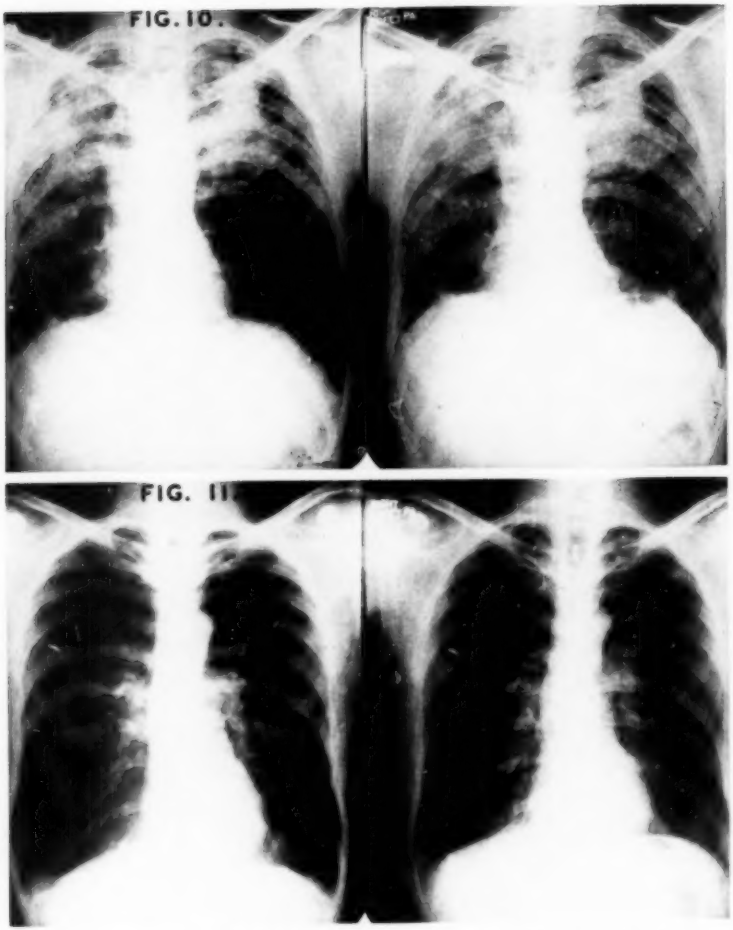


FIG. 10 (Top) Case 5, J. N. Inspiratory and expiratory roentgenograms
FIG. 11 (Bottom) Case 6, J. C. Inspiratory and expiratory roentgenograms

marked cough and expectoration, moderate weakness, and a fifteen pound weight loss during the past ten years. The roentgenographic diagnosis was borderline stage of pneumoconiosis.

Pulmonary Function Studies	Observed	Predicted
Maximal breathing capacity, L. per min.	21	(93)
Vital capacity, cc.	2,050	(3,960)
Residual air, cc.	3,065	(1,700)
Residual air per cent of total lung volume	60.0	(30.0)
Ventilation, L. per min. per Sq.M., B.S.A.		
Rest	3.7	(3.0 to 4.0)
Exercise	—	(8.0 to 11.0)

Pulmonary Function Studies (*cont.*)	Observed	Predicted
Ventilation efficiency, O_2 uptake		
Rest, amount of O_2 removed from air breathed	3.5	(4.5 per cent)
Exercise, amount of O_2 removed from air breathed	—	(5.5 per cent)
Arterial blood oxygen saturation		
Rest	90	(96+ per cent)
Exercise	—	(96+ per cent)
Arterial pCO_2, rest, mm. of mercury	53	(40)
Arterial pO_2, rest, mm. of mercury	64	(95)
Alveolar pO_2, rest, mm. of mercury	81	(100)
Mean gradient, *aeration*, pO_2, inspired air to alveolus, rest, mm. of mercury	66	(50)
Mean gradient, *transfer*, pO_2, alveolus to arterial blood, rest, mm. of mercury	17	(5)
Duration of dyspnea after exercise, sec.	—	(60)

Evaluation: The patient had a far advanced degree of pulmonary function impairment due to: the marked reduction in ventilation, the profound increase in residual air and degree of emphysema, the arterial oxygen unsaturation, the decreased arterial pO_2 and increased arterial pCO_2, and the increased oxygen resting gradients. This patient was too dyspneic at rest to exercise. The decreased ventilation and increased emphysema were the two primary disabling factors in this case.[5]

DISCUSSION

In the present study of 100 anthracite miners, the respiratory gas exchange has been correlated with the degree of pulmonary emphysema. These studies provide physiological data which permit an evaluation of the degree of impairment of pulmonary function present with respect to the transporting of oxygen into, and the removal of carbon dioxide from, the blood by the individual during rest and exercise breathing air. These data, including those previously given (1) may be grouped under four main headings: (*1*) ventilation measurements (maximal breathing capacity and vital capacity); (*2*) estimation of the degree of pulmonary emphysema from quantitative measurements of residual air, and the residual air expressed as per cent of total lung volume; (*3*) determination of the extent of distribution impairment due to unequal alveolar aeration and perfusion with a consequent decrease in arterial pO_2 and per cent saturation; and (*4*) measurement of ventilation efficiency (oxygen uptake from, and carbon dioxide addition to, the air breathed). Each of these four factors may act singly or in combination to impair oxygen transport. Wide individual variations were present.

The physiological classification of emphysema based on the relationship of the volume of residual air to vital capacity and total capacity, and a somewhat arbitrary division of this classification into five categories have been previously reported (1). This quantitative classification of emphysema which is in part, at least, an expression of the effect of the mixing and dilution factor, has proved satisfactory in grouping the cases for study and in analysis of the data. This is

[5] Similar measurements are obtained in cases with extensive pulmonary emphysema with no history of mine exposure or silica inhalation.

shown by the close correlation with other measurements altered by an increased volume of residual air, namely, vital capacity, maximal breathing capacity, effective tidal air, alveolar pO_2, arterial pCO_2, and aeration gradient.

One of the common findings in most cases of anthracosilicosis is the factor of impaired distribution with an increase in the transfer gradient. The impairment results from unequal alveolar aeration and perfusion produced by fibrotic changes. In some alveoli, fibrosis obliterates to a greater or lesser extent the blood flow to the alveolar capillaries resulting in ventilation without perfusion. The alveoli so involved are functional dead space. In other alveoli, fibrosis produces varying degrees of air circulation impairment (loss of lung elasticity and narrowing of the bronchiolar lumen) so that, although perfusion may be present, ventilation is impaired partially or completely. Alveoli which are perfused but nonaerated present circulatory pathways which act as small shunts and lower arterial oxygen saturation. The magnitude of such shunts can be calculated (13, 14). Impaired intrapulmonary mixing of air due to unequal alveolar aeration and perfusion, present in anthracosilicosis, increases the mean gradient of pressure for oxygen from the alveoli to the arterial blood (transfer gradient) and lowers the arterial pO_2.

Evidence obtained by lowering or raising the alveolar pO_2 by breathing low or high oxygen mixtures in nitrogen indicates that the distribution factor is responsible for the changes in the transfer gradient. When the patients were placed on a low level of oxygenation (oxygen per cent of air breathed 11 to 12 per cent or 15 to 18 per cent), the transfer gradient was decreased. However, when the patients were allowed to breathe a 25 to 27 per cent oxygen mixture (high level of oxygenation), the transfer gradient was increased. This method of distinguishing between diffusion difficulty (membrane component) and the distribution difficulty (venous admixture component) has been previously discussed (15). The reverse of the changes described would occur if the difficulty were a true diffusion impairment. The gradient changes are related to the shape of the oxyhemoglobin dissociation curve. In a number of cases, the individual spontaneously elevated the alveolar pO_2 well above 100 mm. of mercury by hyperventilation, thereby increasing the transfer gradient in a manner similar to that of breathing high oxygen mixtures. The arterial pCO_2 decrease, the pH increase, and the aeration gradient decrease were well correlated with the increased alveolar pO_2 in such cases of voluntary hyperventilation.

In most instances, intermittent positive pressure breathing (16) has been found to produce a reduction of elevated transfer gradients even though the alveolar pO_2 was often increased. This lowering of the gradient has been explained on the basis of a more uniform alveolar aeration of those alveoli which were poorly ventilated on ambient breathing but were still perfused with blood.

A good correlation has been reported in chronic pulmonary emphysema between the degree of emphysema and arterial oxygen unsaturation (17). In most of the anthracosilicotic cases studied, however, the resting arterial oxygen saturation was moderately reduced but poorly correlated with pulmonary function impairment. A single resting arterial blood saturation measurement had but

limited value unless it was extremely low, because wide individual variations were present but accompanied by only slight average changes. Measurements of the arterial oxygen saturation immediately following the step-up exercise revealed significant differences with respect to the degree of emphysema and the existing disability. The changes in arterial oxygen saturation after exercise were closely correlated with the degree of emphysema in most cases. In a few patients, however, the arterial oxygen saturation increased slightly after exercise even in the presence of extensive emphysema, and in others the saturation decreased even in the absence of an increased residual air. The latter variation was the result of marked inequality in alveolar aeration and perfusion. There was no apparent means of predicting in advance the effect of exercise on the arterial oxygen saturation with respect to the resting measurements. The exercise saturation measurement has proved to be one of the most significant points in evaluating pulmonary function impairment in anthracosilicosis.

Acute hypoxia was found to elevate pulmonary artery pressure in essentially normal individuals and in individuals with chronic pulmonary disease (10). Studies on the pulmonary circulation during exercise revealed definite abnormalities in chronic pulmonary disease, including a rise in pulmonary artery pressure and an increase in pulmonary vascular resistance as compared to unchanged or lowered pulmonary artery pressure during exercise in normal subjects (18, 19). Acute hypoxia may also contribute to the elevation of pulmonary artery pressure in patients with a greater degree of arterial oxygen unsaturation during exercise.

In patients with anthracosilicosis, the presence of polycythemia of a significant degree has been a rare finding. Even though a considerable number of the patients with a far advanced degree of pulmonary emphysema exhibited marked reduction in ventilatory capacity, a high arterial pCO_2, a low arterial pO_2 with arterial oxygen unsaturation and marked dyspnea on slight exertion, no polycythemia or elevation of the hemoglobin level was present. Somewhat similar findings on polycythemia have been reported in chronic pulmonary granulomatosis of beryllium workers (20) who have a marked lowering of arterial pO_2.

The arterial pO_2 was decreased in most cases with only a slightly larger average fall in the respectively greater degrees of emphysema. The arterial pO_2 was a more sensitive index of early changes than the per cent saturation because there is a wider spread in the upper flat portions of the oxyhemoglobin dissociation curve. The arterial pCO_2 values were correlated with the degree of emphysema as shown by the decreased ability to remove carbon dioxide in the presence of increased residual air. In a few patients, however, the hyperventilation was of a sufficient magnitude to lower the pCO_2 value to 40 mm. of mercury. Although the arterial pCO_2 values were markedly elevated in some cases with extensive emphysema, the blood pH measurements indicated that the patients were acclimated to the higher levels.

The ventilation efficiency of the lung as measured by the oxygen uptake or carbon dioxide output had a limited significance in the evaluation of pulmonary function impairment in anthracosilicosis. The removal of oxygen per given

volume of air breathed was below the normal level in the majority of the coal miners studied. In some cases, most often in patients with extensive emphysema, the amount of oxygen removed during exercise per volume of air breathed was less than during rest. Measurements of the amount of carbon dioxide removed from the blood were more or less parallel to those noted for oxygen. In general the studies of expired air showed that the oxygen removal and carbon dioxide addition were subnormal in almost all cases, although wide individual variations occurred and only slight average differences existed between the degrees of pulmonary function impairment. If the oxygen uptake decreased markedly with exercise, this measurement was significant for the individual case. Only slight information was provided, however, as to the nature of the impairment other than to indicate the existence of a disproportion pertaining both to the intrapulmonary mixing of inspired air and the pulmonary blood flow. The oxygen uptake and carbon dioxide output determinations were useful for evaluation with the other tests, but these measurements should not be used alone.

At the present time studies on pulmonary function impairment are being extended by making measurements during a steady state of exercise provided by a treadmill; also, right heart pressures with cardiac output determinations are being obtained. The pressure in the pulmonary artery may be normal or elevated in chronic pulmonary disease. With exercise, however, the increase in cardiac output may be much less than normally expected and the pulmonary artery pressure may be elevated from the resting level and pulmonary vascular resistance increased. These hemodynamic changes limit the transfer of oxygen to, and the removal of carbon dioxide from, the tissues, and may be the major factor responsible for pulmonary disability in some patients. A prolongation of dyspnea (3 to 5 minutes) following the step-up exercise for one minute suggests that the pulmonary circulation may be the primary factor in producing disability when measurements on ventilation, residual air, and arterial blood gases show slight to moderate abnormality.

SUMMARY

Physiological measurements pertaining to respiratory gas exchange have been obtained in 100 unselected anthracite coal miners who had pulmonary symptoms and a long history of exposure to mine dust. These measurements have been correlated with the degree of pulmonary emphysema.

In approximately one-third of the cases of anthracosilicosis studied, the residual air per cent of total lung volume was not significantly increased. The disability present in these cases arose from fibrosis and the accompanying unequal alveolar aeration and perfusion, which decreased the amount of oxygen in the arterial blood (the distribution factor). The distribution factor was important in most cases of anthracosilicosis regardless of the degree of emphysema. The present studies indicated that usually the difficulty in the transfer of gases between the alveoli and the blood results from poor distribution rather than a true diffusion impediment with increased resistance at the pulmonary membrane.

A marked decrease in the arterial oxygen saturation after exercise was a very

significant finding in evaluation of disability because there was a greater disproportion between the ventilation and perfusion ratios occurring with the increased oxygen consumption during work as compared to that during rest. The resting arterial oxygen saturation alone was poorly correlated with pulmonary function impairment as the respiratory gas exchange measurements might be essentially normal at rest, although during exercise gross abnormalities might be manifest.

Determination of the per cent of oxygen uptake and carbon dioxide addition to the air breathed (or expressed as respiratory equivalents) was helpful in the over-all evaluation of the impairment present, but this test had slight value as a single measurement and should not be used alone.

Polycythemia or elevation of the hemoglobin level were rare findings even in the presence of advanced emphysema, reduced ventilatory capacity, arterial oxygen unsaturation, and marked dyspnea on exertion.

Pulmonary function data and physiological evaluation are given for typical cases with various degrees of pulmonary function impairment.

An accurate evaluation of the degree of pulmonary function impairment requires measurements of: ventilation, residual air, oxygen uptake from the air breathed, and arterial blood studies during both rest and exercise. In selected cases, measurements on the pulmonary circulation are necessary. The wide individual variations encountered in these determinations preclude the use of any one single test for the satisfactory appraisal of disability in all types of cases.

SUMARIO

Estudios del Intracambio Respiratorio de Gases en Cien Mineros de Antracita que Mostraban Síntomas Pulmonares

En 100 casos tomados al azar de mineros de antracita que tenían síntomas pulmonares y larga historia de exposición al polvo de las minas, obtuviéronse mediciones fisiológicas relativas al cambio respiratorio de gases, que luego se correlacionaron con la intensidad del enfisema pulmonar.

Aproximadamente en la tercera parte de los casos de antracosilicosis estudiados, no aumentó mayor cosa el por ciento de aire residual por volumen pulmonar total. La incapacidad presente en esos casos dimanaba de la fibrosis y de las desiguales aeración y perfusión concomitantes, que hacían disminuir la cantidad de oxígeno en la sangre arterial (el factor de distribución). Este factor resultó importante en la mayor parte de los casos de antracosilicosis independientemente de la intensidad del enfisema. Los estudios actuales indicaron que, por lo general, la dificultad con que tropieza el pase de gases entre los alvéolos y la sangre proviene de mala distribución, más bien que de verdadero impedimento a la difusión con hiperresistencia en la membrana pulmonar.

Una pronunciada disminución de la saturación arterial de oxígeno después del ejercicio constituyó un hallazgo muy significativo en la justipreciación de la incapacidad por ser mayor la desproporción entre las proporciones de ventilación y perfusión al aumentar el consumo de oxígeno durante el trabajo, comparada con la observada durante el descanso. La saturación arterial de oxígeno en reposo

por sí sola estaba poco correlacionada con la disfunción pulmonar, dado que las mediciones del cambio respiratorio de gases podían ser esencialmente normales en descanso, aunque mostrando importantes anomalías durante el ejercicio.

La determinación del porcentaje de toma del oxígeno y de la adición de bióxido de carbono al aire respirado (o su expresión en equivalentes respiratorios) resultó útil para la valuación global de la incapacidad presente, pero esta prueba mostró poco valor como medición aislada y no debe usarse por sí sola.

La policitemia o la elevación del tenor de hemoglobina constituyeron hallazgos raros aun en presencia de enfisema avanzado, reducción de la capacidad ventiladora, inoxigenación arterial y pronunciada disnea de ejercicio.

Preséntanse datos de la función pulmonar y valuación fisiológica para típicos casos con varios grados de disfunción pulmonar.

La justipreciación de la intensidad de la disfunción pulmonar exige mediciones de: la ventilación, el aire residual, la toma de oxígeno del aire respirado, y estudios de la sangre arterial tanto durante el descanso como durante el ejercicio. En ciertos casos, necesítanse mediciones de la circulación pulmonar. Las amplias variaciones individuales observadas en estas determinaciones excluyen el empleo de una prueba aislada para la valuación satisfactoria de la incapacidad en todo género de casos.

REFERENCES

(1) Motley, H. L., Lang, L. P., and Gordon, B.: Pulmonary emphysema and ventilation measurements in one hundred anthracite coal miners with respiratory complaints, Am. Rev. Tuberc., 1949, *59*, 270.

(2) Riley, R. L., Proemmel, D. D., and Franke, R. E.: A direct method for determination of oxygen and carbon dioxide tensions in blood, J. Biol. Chem., 1945, *161*, 621.

(3) Van Slyke, D. D., and Neill, J. M.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurement, J. Biol. Chem., 1924, *61*, 523.

(4) Roughton, F. J. W., Darling, R. C., and Root, W. S.: Factors affecting the determination of oxygen capacity, content and pressure in human arterial blood, Am. J. Physiol., 1944, *142*, 708.

(5) Sendroy, J., Jr.: Manometric determination of hemoglobin by the oxygen capacity method, J. Biol. Chem., 1931, *91*, 307.

(6) Riley, R. L., Lilienthal, J. L., Jr., Proemmel, D. D., and Franke, R. E.: On the determination of the physiologically effective pressure of oxygen and carbon dioxide in alveolar air, Am. J. Physiol., 1946, *147*, 191.

(7) Scholander, P. F.: Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples, J. Biol. Chem., 1947, *167*, 235.

(8) Gordon, B., Motley, H. L., Theodos, P. A., and Lang, L. P.: Anthracosilicosis and its symptomatic treatment, West Virginia M. J., 1949, *45*, 126.

(9) Theodos, P. A., Gordon, B., Lang, L. P., and Motley, H. L.: The clinical evaluation of disability in anthracosilicosis, Dis. of Chest, 1949 (in press).

(10) Motley, H. L., Cournand, A., Werko, L., and Dresdale, D.: The influence of short periods of induced acute anoxia upon pulmonary artery pressures in man, Am. J. Physiol., 1947, *150*, 315.

(11) Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency: I. Discussion of a physiological classification and presentation of clinical tests, Am. Rev. Tuberc., 1941, *44*, 26.

(12) Baldwin, E., Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency: I. Physiological classification, clinical methods of analysis, standard values in normal subjects, Medicine, 1948, *27*, 243.

(13) Riley, R. L., and Cournand, A.: Analysis of ventilation perfusion relationships in the lungs, Federation Proc., 1949, *8*, 132.

(14) Rahn, H.: Effect of unequal blood flow and ventilation upon the alveolar-arterial gradient, Federation Proc., 1949, *8*, 129.

(15) Lilienthal, J. L., Jr., Riley, R. L., Proemmel, D. D., and Franke, R. E.: An experimental analysis in man of the oxygen pressure gradient from alveolar air to arterial blood during rest and exercise at sea level and at altitude, Am. J. Physiol., 1946, *147*, 199.

(16) Motley, H. L., Lang, L. P., and Gordon, B.: The influence of intermittent positive pressure breathing on the respiratory gas exchange, J. Aviation Med., 1949 (in press).

(17) Hurtado, A., Kaltreider, N. L., and McCann, W. S.: Studies in total pulmonary capacity and its subdivisions: VI. Relationship to the oxygen saturation and carbon dioxide content of the arterial blood, J. Clin. Investigation, 1935, *14*, 94.

(18) Riley, R. L., Himmelstein, A., Motley, H. L., Weiner, H. M., and Cournand, A.: Studies of the pulmonary circulation at rest and during exercise in normal individuals and in patients with chronic pulmonary disease, Am. J. Physiol., 1948, *152*, 372.

(19) Hickam, J. B., and Cargill, W. H.: Effect of exercise on cardiac output and pulmonary arterial pressure in normal persons and in patients with cardiovascular disease and pulmonary emphysema, J. Clin. Investigation, 1948, *27*, 10.

(20) Bruce, R. A., Lovejoy, F. W., Brothers, G. B., and Velasquez, T.: Observations on the cause of dyspnea in chronic pulmonary granulomatosis in beryllium workers, Am. Rev. Tuberc., 1949, *59*, 364.

PARA-AMINOSALICYLIC ACID TREATMENT OF TUBERCULOSIS[1]

A Review

EMIL BOGEN,[2] RUSSELL N. LOOMIS, AND DRAKE W. WILL

(Received for publication July 1, 1949)

HISTORICAL BACKGROUND

The intelligent recognition of an incidental discovery in the course of an unrelated investigation is illustrated in the development of the para-aminosalicylic acid treatment for tuberculosis. Although the original assumption of Bernheim and Lehmann, that materials which stimulate the oxygen consumption of tubercle bacilli would favor its growth and that interference with this mechanism would inhibit the growth of the bacilli, was incorrect, the careful and intelligent investigation of this hypothesis led to one of the most promising discoveries in the treatment of tuberculosis.

That tubercle bacilli grow and multiply only in the presence of oxygen, water, minerals, and nitrogenous and carbon compounds was well established soon after the discovery of the bacillus. Simple synthetic media facilitated investigation of the stimulating and retarding effects of other agents. Although respiratory studies of tubercle bacilli had been reported previously, the development of the microrespiration apparatus of Warburg led to more precise observation of the effects of various substances on oxygen consumption by the bacilli.

Glycerol, lactic acid, and other agents were demonstrated to accelerate this oxygen consumption. In 1940, Bernheim reported that benzoic acid and salicylic acid greatly increased the oxygen consumption of tubercle bacilli while many closely related substances did not. He concluded that benzoic and salicylic acid were oxidized by the tubercle bacillus and that similar chemical configurations play a part in its normal metabolism, and suggested that other derivatives might have a reverse effect.

The stimulating effect of benzoic and salicylic acid on the metabolism of pathogenic virulent human-type tubercle bacilli was soon confirmed, but Lehmann found that this was not true of nonpathogenic strains such as BCG. Lehmann also tested a large number of derivatives of benzoic acid in the hope of finding one which would combine with the bacillus in the same manner as does benzoic acid without being utilized by it and thus block one of the metabolic processes of this organism. The ability of benzoic and salicylic acid to stimulate the oxygen consumption of the tubercle bacillus was found, however, to be accompanied by inhibition of the growth and multiplication of the bacilli. The actual testing of the derivatives accordingly was made by noting the inhibitory effect of their presence on the growth of the tubercle bacilli *in vitro*. Although BCG did not

[1] This study was aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service.

[2] Olive View, California.

show the increased oxygen consumption from benzoic acid which Bernheim had reported, it was with this organism that Lehmann proceeded to carry out his productive investigations.

Systematic investigation of a large series of substitution products of benzoic acid showed that the addition of a hydroxyl group, or of an amino group, to the benzoic acid radical increased its growth-inhibiting effect on the tubercle bacillus, and that the position in which these radicals were attached was of great importance. Thus, of the various hydroxy derivatives of benzoic acid, the ortho-hydroxy-benzoic acid, salicylic acid, was most effective in inhibiting the growth of BCG, while the meta-hydroxy-benzoic acid showed less effect, and the para-hydroxy-benzoic acid the least. On the other hand, of the amino derivatives of benzoic acid, meta-amino-benzoic acid caused less inhibition of the growth of these organisms and ortho-amino-benzoic acid the least. When these radicals were combined, the para-4-amino-ortho-2-hydroxy benzoic acid was found to be far more effective in inhibiting the growth of tubercle bacilli than any of the other possible combinations. It was not until the end of 1943 that Rosdahl, a Swedish chemist, was able to prepare para-aminosalicylic acid for Lehmann. Within a few months thereafter, Lehmann had performed sufficient test tube and animal experiments to warrant clinical application. Thus, in the middle of the year 1944, para-aminosalicylic acid was first administered to man as treatment for tuberculosis.

PREPARATION

Para-aminosalicylic acid was first reported by Seidel and Bittner as having been prepared by the reduction of nitrosalicylic acid. This procedure is suggested by reports on the earlier German patents. A number of studies have been published on material prepared by this method. Ghielmetti, however, insists that the yield of this procedure is actually meta-amino-phenol, an entirely different material which is more toxic and lacks the tuberculostatic properties of para-aminosalicylic acid. Gulinov in Russia, Rosdahl in Sweden, Erlenmeyer in Switzerland, Justoni in Italy, Seymour in England, and Sheehan in America described other methods for the production of para-aminosalicylic acid and for its purification. Many of these methods require involved oxidations or reductions under greatly increased pressures and the use of special reagents.

Although PAS has thus been produced by a dozen different workers abroad and by an equal number of American firms, and patents have been issued for a variety of procedures for its preparation, there is still great difficulty in obtaining adequate supplies of pure material. This is due to the scarcity of sufficiently pure reagents or precursors used in its manufacture, to inconstancy of yields in the processes of manufacture, to difficulty in removing all impurities from it, and to the fact that PAS decomposes readily. Because of the instability of the material and the difficulty of obtaining pure PAS, great discrepancies appear in the literature regarding its physical, chemical, pharmacological, and therapeutic properties.

PHYSICAL PROPERTIES

PAS is a white, crystalline powder of low specific gravity. It decomposes on application of heat. This has given rise to difficulties in the determination of its melting point. Most authors report the melting point of PAS to be between 137° C. and 160° C. and the majority of the specimens tested here have melted, on rapid heating, at about 145° C. There is too great a difference to be ascribed merely to the usual effect of impurities in raising the melting point. The most prominent degradation product, meta-amino-phenol, melts at a lower temperature, about 123° C. However, the hydrochloride of PAS melts at 220° to 225° C. and so the higher values probably represent the results of studies of the acid salts of PAS. The more slowly the melting point determination is made, the greater will be the error, since on such slow heating a greater amount of decomposition occurs with consequent change of the melting point. On rapid exposure to heat, particularly in the absence of oxygen, it appears that the melting point is probably in the neighborhood of 145° C., but it is difficult to obtain close checks on successive determinations of apparently similar samples, or different batches of the same product, or the products of different companies. Nevertheless, it may be suspected that any product whose melting point differs markedly from 145° C. is not a pure PAS.

Although pure PAS is white and forms a colorless solution in water, many commercial preparations show varying amounts of color, from light cream or brownish yellow to a deep brown or gray, or occasionally a reddish or bluish tint. The brown color apparently results from decomposition or betrays impurity, and decomposition in solution is generally evidenced by a deepening in the intensity of the brown color, but is not proportional to it.

The taste of PAS is mildly sour but not very unpleasant or acrid. Most commercial preparations of it possess a bitter taste or after-taste suggestive of aspirin or quinine, making it quite disagreeable, particularly with certain of the preparations whose after-taste lingers and may even nauseate. Part of this bitterness may be due to meta-amino-phenol. Although phenol is itself strongly bacteriostatic against the tubercle bacillus, it is far too toxic for internal use in man. The meta-amino-phenol, though less toxic than phenol, apparently has very little action against the tubercle bacillus.

PAS is very slightly soluble in distilled water, about 0.5 per cent, or in acid solutions, but it is moderately soluble in alcohol or ether, and readily soluble in alkaline solutions forming the highly soluble monosodium salt. It is reported that PAS is more soluble in urine than in water, rising from less than one per cent when the pH is 4, to more than 15 per cent at pH 7 and thus the risk of precipitation in the kidneys is practically negligible. The hydrochloride of PAS which is even less water-soluble has a melting point between 220° to 225° C., and may be somewhat more stable than the free base. This salt has so far received relatively little attention in the literature and is not generally used or available. The monosodium salt, on the other hand, is soluble up to 95 Gm. in 100 cc. of water and insoluble in ether. It yields a neutral solution, rapidly absorbed, with a somewhat bitter taste. It melts and decomposes above the melting

point of the hydrochloride and is more stable than the free acid in solution, but more data regarding it should be obtained.

The resultant decarboxylation and production of meta-amino-phenol and other products not only lowers the amount of PAS and darkens the solution to a brownish color, but produces a change in taste and especially irritative properties on the gastrointestinal tract on ingestion which lessens the tolerability of such preparations. Decomposition, especially decarboxylation with the liberation of meta-amino-phenol, occurs readily under the influence of moisture, light, air, heat, or simple standing, especially when exposed to air. Accordingly it is advised that supplies of PAS should be kept in the dark or in light-proof containers, under refrigeration, and sealed so far as possible from exposure to moisture or the oxygen of the air Although it has generally been observed that aqueous solutions of para-aminosalicylic acid, or the more concentrated neutralized acid or monosodium salt, gradually darken in solution even at room temperature and that this change is accelerated at higher temperature, some authors have recommended that the solutions may be made up and kept for some time (as long as a month), while others sterilize the solution by autoclaving. The decarboxylation and decomposition of the para-aminosalicylic acid solutions can be retarded if they are sealed from air and kept under pressure while being heated. It appears preferable when possible, as other workers have advised, to sterilize by filtration through a Berkefeld, or Seitz, or similar filter. For some purposes we have found it preferable to sterilize simply by treating the dry sodium PAS powder with 75 per cent ethyl alcohol and shaking, and after that allowing it to settle and removing the alcohol before making up the solution under sterile conditions. If solutions are made up beforehand, they should be kept in a dark container at low temperature and tightly sealed in containers that are completely filled, to prevent oxidation or the loss of CO_2.

More than 60 different preparations of PAS, produced by a dozen different manufacturers both in the United States and abroad, have been examined at Olive View. These preparations have borne various designations, such as: para-aminosalicylic acid, PAS, para-amino-ortho-hydroxy-benzoic acid, 4 amino-salicylic acid, 4 amino-ortho-2-hydroxy-benzoic acid, Pamasyl, Pamasil, Paramisal, Pamisyl, Parasal, et cetera.

The retail price of PAS has varied from $10 to $40 a pound for different preparations and at different times but most of the PAS used for therapy in these studies was kindly donated by the Parke, Davis & Company, the Rexall Drug Company, and the Hyland Laboratories. It is hoped that cheaper, as well as purer, preparations may become available in the near future.

Most preparations of PAS are almost completely destroyed and volatilized on combustion, but a few leave varying amounts in combustible ash. Several PAS salts contained chlorides or sulfates, but all the others yielded negative tests for these substances. The powders are practically anhydrous as received and are not hygroscopic under ordinary conditions. The pH of fresh saturated solutions, as tested, was usually about 3.2 but rose to over 4.5 on standing.

Physical, chemical, and biological tests have been performed, both in the

test tube and in experimental animals to compare their properties. A great variety in color, taste, consistency, weight, solubility, melting point, and other physical properties indicates the variability of this product even though simple chemical tests indicate that the impurities represent only a small per cent of the material.

The dry materials were generally fine crystalline powders, although several were amorphous. They were usually very light and fluffy, one gram occupying from 1.5 to 4 cc. in a graduate when not pressed down, but it could be compressed to less than 0.5 cc. The material varied in color from white or colorless through cream, yellowish-gray, or brown; but pinkish, purplish, lavender, and buff shades were also noted. Solutions were usually colorless, yellowish, or brown with an occasional light purplish cast and darkened on standing. The colors did not seem to be proportional to their bacteriostatic effectiveness. In general, however, the darker preparations were more bitter and distasteful.

The powders, or their solutions, were odorless but had, generally, a slightly sour taste. One was highly sour while several were only very slightly sour and a few showed only a flat, chalky, or dull charcoal insipidity. Most of them also had a disagreeable aspirin-like bitterness or after-taste of varying intensity and a few showed salty, alkaline, or other components. The taste was lessened or partially covered by partial neutralization or the addition of soda, sugar, or flavors, but was usually still unpleasant.

There were also differences in solubility and in the pH and titratable acidity of the saturated and .07 per cent solutions. Most of them appeared to be soluble only to the extent of about .12 per cent and gave saturated solutions with a pH of about 3.2. Titration with NaOH for total acidity or with $FeNO_3$ solution for total salicylate or with the Ehrlich diazo reagent for amines, however, indicated that most of the preparations were more than 95 per cent PAS; the wide discrepancies in their behavior being due to the presence of comparatively small amounts of impurities.

Melting points varied widely and were accompanied by different amounts of decomposition shown by darkening of color and the evolution of gas, probably CO_2. The standard melting point test of slow heating in capillaries in a glycerin bath, and more rapid heating both in capillaries and on a metal plate, were used and gave different results. Some melting points below 130° C. and several above 220° C. were noted, but the latter were found in preparations yielding positive tests for chloride or sulfate or sodium ions and probably represent the corresponding salts. Most of the preparations melted at about 138° to 148° C. on rapid heating, although it is difficult to check because of associated decompositions.

TUBERCULOSTASIS IN VITRO

PAS inhibits the growth of the avirulent, attenuated bovine strain BCG growing on Sauton's synthetic liquid medium, although the oxygen consumption of the BCG is apparently not accelerated by benzoic or salicylic acid as it is with virulent organisms. This inhibitory effect is bacteriostatic, not bactericidal, and

does not affect the staining properties of the tubercle bacillus. With the exception of BCG, all other avirulent, acid-fast strains and other nonpathogenic strains studied by Lehmann and others were not affected by PAS at low concentrations. All other microorganisms so far examined, with the exception of virulent tubercle bacilli and BCG, are also resistant to PAS. PAS, prepared by Dr. Rubin, was reported to have no effect against *Escherichia coli* by Wyss as early as 1943. Dozens of different cultures studies by Sievers and many organisms examined by other workers all proved resistant to PAS. On the other hand, six virulent human type tubercle bacilli strains studied orginally by Lehmann, and hundreds of virulent tubercle bacilli tested since, have proved almost uniformly susceptible to the effect of PAS. One bovine strain and one avian strain were reported to be sensitive to PAS by Youmans, although it has been said that other bovine type tubercle bacilli may be resistant as well as the avirulent human bacilli.

Tests of bacteriostatic properties of PAS against tubercle bacilli have been performed on Sauton's, Dubos, and Kirchner's synthetic liquid media with and without the addition of albumin and blood serum or Tween. Similar tests have also been performed on solid media, with the use of filter-paper disks as are used in penicillin assay, or on slides by a modified Pryce culture technique. Low concentrations of PAS produce only partial inhibition of the growth of the tubercle bacillus and even higher concentrations often yield incomplete bacteriostasis in the hands of most workers. The proportion of bacilli inhibited varies from below 50 per cent to over 95 per cent in different reports. The concentrations of PAS necessary to inhibit the growth of tubercle bacilli vary from one to 10 γ per ml. in the case of different strains of tubercle bacilli and under different conditions.

The inhibitory effect is apparently not lessened by the presence of serum and may even be increased by the presence of Tween 80, but the inhibition is markedly decreased by increasing the number of tubercle bacilli in the inoculum and may even be missed entirely with heavy inoculation. Readings should be made at about one week after inoculation since the inhibitory property may not be in evidence during the first few days after the inoculation and may be overcome by continued growth of the small proportion of survivors if the cultures are incubated for too long a time. Although some reports of organisms resistant to PAS have been based on studies of growth on solid media, most of the reports of uniform susceptibility have resulted from experiments on liquid media. Our own findings indicate that the results on these two types of media are generally fairly similar if small inocula are used.

It is reported that the addition of PAS may partially and inconstantly inhibit the increase of oxygen consumption by tubercle bacilli resulting from the presence of benzoic or salicylic acid, while the addition of benzoic or salicylic acid may inhibit the stimulating effects of PAS. The inhibitory effect of PAS on the growth or multiplication of tubercle bacilli, as with sulfonamides, may be partially stopped or even reversed by the presence of para-amino-benzoic acid, according to some, but not all, workers. Youmans states that inhibition of

growth of tubercle bacilli by PAS is not affected by the presence of sodium salicylate, in spite of the apparent interaction of these agents on oxygen consumption.

How PAS interferes with the tubercle bacillus is still uncertain. Lehmann points to the work of Herner who found that benzoic and salicylic acids inhibit specifically the deamination of amino acids. Inhibition of oxidation of the benzoic or salicylic acids themselves is suggested by Bernheim's early work. The stimulation of respiration by PAS observed by Lehmann may itself inhibit the growth of the organisms. Other mechanisms are still to be investigated.

Some of the differences in results reported by different workers in *in vitro* experiments may be explained by the observations of Duca who found that one preparation of PAS was only one-eighth as potent in inhibiting the growth of tubercle bacilli as another preparation obtained from the same manufacturer. In our own experiments, trial of different preparations labeled para-aminosalicylic acid against various strains of tubercle bacilli, in liquid and solid media, showed that most of them were tuberculostatic but none bactericidal and that their effectiveness was generally detectable at about 1.0 γ per ml. concentration, although it differed with different products.

PAS hydrochloride and the sodium salt of PAS have apparently approximately similar effects on the growth of tubercle bacilli as the free base. It is more convenient to use the sodium salt in *in vitro* experiments, owing to its solubility. Although benzoic acid and salicylic acid increase the oxygen consumption of tubercle bacilli and may partially inhibit the growth of the organisms *in vitro*, animal experiments, including those at Olive View, fail to show therapeutic value of these agents in the treatment of experimental tuberculosis. Ethyl benzoic acid or benzocaine or Anesthesin, as it is commercially known, and isobutyl benzoic acid or Cycloform were found by Bloch to be similarly effective in inhibiting the growth of tubercle bacilli *in vitro* but are too toxic to warrant administration in adequate dosage. Numerous other derivatives of benzoic acid and related substances have been tested and found to have only slight or no effect on the growth of tubercle bacilli or on their respiration.

TOXICITY IN ANIMALS

Although PAS is generally consistently reported as inhibiting the growth of the tubercle bacillus *in vitro*, when administered in adequate concentration to a sufficiently small inoculum of bacteria, there have been considerable discrepancies in the reports of animal experimentation. Much of this variation has been due to differences in dosage utilized in the experiments. This, in turn, has been necessitated by the greatly different toxicity in the preparations used by various workers. Thus, working with mice, Lehmann reported that he found no toxic effect at all when he administered PAS in 5 per cent concentration in their diet, while Youmans found toxic effects when 4 per cent PAS was given in the mouse diet but none with 2 per cent. Rats were reported as unaffected by 5 per cent PAS in their diet or by 2 Gm. per Kg. Lehmann reported that 0.1 Gm. per Kg., in 10 per cent solutions administered intramuscularly, intravenously, and orally

produced no reaction in rabbits. McClosky reported that rabbits tolerated 0.5 Gm. per Kg. for the most part but, in the case of one who died after thirty-six days of treatment, the death was attributed to the toxicity of the drug.

Greater discrepancies have been observed in the experiments with guinea pigs. Lehmann found that 5 per cent PAS caused loss of appetite, weight, and growth and a syndrome simulating B avitaminosis with emaciation, loss of hair, and large hemorrhagic adrenal glands on autopsy. Feldman found that his guinea pigs tolerated 4 per cent PAS in their diet, or 1.6 Gm. daily, but McClosky, whose guinea pigs tolerated 2.0 Gm. per Kg. in a single dose, showed a 30 per cent mortality when 0.5 Gm. was administered daily.

In our own experience, rabbits have tolerated 10 Gm. of PAS orally, daily, for many months while guinea pigs have similarly taken 2.0 Gm. of PAS per day with impunity but refuse to eat feed containing larger amounts, or concentrations greater than 5 per cent in the rolled grain.

Intradermal and subcutaneous injections of 0.1 cc. and 1.0 cc., respectively, of neutralized solutions $\left(\frac{N}{10}\right.$ and 20 per cent at a pH about 7$\left.\right)$ showed marked variation in the intensity of local reaction. Several preparations resulted in severe local necrosis, others produced marked edema, while others caused only slight temporary redness or no reaction. Intravenous infections of 10 cc. of a 20 per cent solution were tolerated by rabbits even when repeated several times daily.

THERAPEUTIC EFFECTS ON EXPERIMENTAL TUBERCULOSIS

Youmans and his co-workers have found PAS highly effective in the treatment of experimental tuberculosis in mice, and Moeschlin has had similar findings. Swedberg reported both successful and unsuccessful experiments. McClosky found only a slight suppressive effect of 0.5 mg. per Kg. daily and concluded that the treatment was of little value.

Lehmann was unable to show benefit from the administration of 5 per cent PAS in the feed of guinea pigs and Feldman's first experiment, using 2 per cent to 3 per cent in the feed for forty-eight days, also yielded questionable results. McClosky found low, if any, activity in treatment with 5 per cent or less in the feed. Swedberg also questioned the results that he obtained in some experiments with guinea pigs. However, Feldman, McClosky, and Swedberg actually did report some successful experiments with this material.

Experiments at Olive View have shown that dosage of 0.1 Gm. per day to a guinea pig is ineffective and 0.5 Gm. per day gives only a partial result while 2.0 Gm. daily gave still better results than 1.0 Gm. dosage. Similarly, when PAS was administered at intervals with treatment once a week, it gave very little result while treatment three times a week, 2.0 Gm. each dose, was markedly effective. Various products of PAS also gave differences in effectiveness, due in part to the differences in palatability and the amounts of the material which the guinea pigs took in their food. Effective amounts of PAS could not be administered subcutaneously in guinea pigs, but could be given intravenously in

rabbits. Combinations of PAS with small amounts of streptomycin had a more marked effect than either alone, but this synergistic effect was not observed with several sulfones or with tuberculin. The simultaneous administration of an equal amount of para-amino-benzoic acid did not prevent the favorable action of PAS in a cage of tuberculous guinea pigs.

PHARMACOLOGY OF PAS

Para-aminosalicylic acid may be determined in pure concentrated solutions simply by titration for acidity, or by the use of the iron chloride test solution, which gives a wine red color with salicylates, but more delicate procedures are required for determination of the small amounts found in blood or tissues. Ehrlich's diazo reagent yields a color with PAS which is widely used for PAS determination in body fluids but is nonspecific and yields a blank sometimes as high as one milligram per 100 cc. in the absence of administration of PAS. Consequently, this large blank must be subtracted from the results of the assay. The Bratton and Marshall technique, similar to that used with sulfonamides, is also used as it gives greater precision but it is technically somewhat more difficult. Para-aminosalicylic acid produces fluorescence and this or its ultraviolet absorption spectrum may be utilized for its determination with the use of a spectrophotometer.

PAS is rapidly absorbed from the gastrointestinal tract. The rate of absorption depends partly on the physical form administered, solutions of the sodium salt most rapidly reaching the blood stream. The acid preparations, particularly within granules or compressed tablets and when administered with an enteric coating such as salol, stearine, or keratin, may be much more slowly absorbed. The salol, however, may itself be toxic while excessive coating, especially in the presence of diarrhea from the drug, may sometimes prevent absorption and the pills appear intact in the stools. Neutral solutions of PAS or the sodium salt may also be administered parenterally, intrapleurally, or intravenously but are generally too irritating for subcutaneous or intramuscular or intraspinal administration.

PAS, after absorption, diffuses freely throughout the tissues, reaching concentrations in the pleural fluid and other body fluids similar to that in the blood. There is no apparent storage of the material after it disappears from the blood stream. It has been reported, however, that PAS is not to be found in the cerebrospinal fluid or exudate. Blood levels achieve a range from mere traces to 14 mg. per 100 cc. or 140 γ per ml., depending on the dosage and manner of administration, but most investigators report concentrations between one and 10 mg. per 100 cc. after effective oral administration. Although 1.0 mg. per 100 cc. is generally bacteriostatic *in vitro*, it may be that higher peak levels are required *in vivo* to yield therapeutic effects. The concentration of PAS in the blood following single oral administrations, however, rapidly diminishes and so the material must be administered frequently, *e.g.*, every three hours, to maintain continued levels, unless slowly absorbed material is administered. Such continuous levels may not be necessary, however, to obtain therapeutic response.

Most of the PAS is excreted in the urine; 85 per cent has been reported as recovered in the urine within ten hours after administration. Part of it may be recovered as free PAS acid or the sodium salt. Another part is combined or changed so that it loses its bacteriostatic property. Most of the changed PAS in the urine appears in the form of the acetyl compound, acetyl-aminosalicylic acid although para-aminosalicyluric acid and acetylated para-aminosalicyluric acid are also reported. Some deaminization may occur and some 2-4-dihydroxy-benzoic acid has been reported. It appears, however, that meta-amino-phenol, a common degradation product *in vitro*, and the glycuronamide so commonly resulting from the neutralization of other agents in the liver do not appear in the urine, at least not in the urine of man, according to several workers. Some PAS may be more completely destroyed within the body but little is known regarding the extent, or the paths, of such destruction. It has been reported that 90 per cent of the PAS is excreted unchanged by the rat, while 60 per cent is conjugated or acetylated in man; but other workers have found different proportions.

RESISTANT STRAINS

The development of strains of tubercle bacilli which are resistant to the effect of PAS has been reported by a number of workers. There is some difference in the reports regarding this. It is apparently much more difficult to obtain strains resistant to PAS than to streptomycin. Lehmann and Alin have not encountered such organisms but the fact that they may be produced *in vitro* has been reported by Hobby and by Dye. The production of resistant strains in animals infected experimentally by treatment with PAS has not yet been reported. Several workers reported finding strains of tubercle bacilli partially resistant to PAS in patients treated with this material, but others deny this. Lehmann has attributed some of the reports to the finding of avirulent acid-fast saprophytes, bovine-type tubercle bacilli, or other organisms which, even without treatment, would have been resistant to PAS.

Both *in vitro* and in animal experiments, moreover, as well as in clinical experience, PAS exerts its effect just as strongly on streptomycin-resistant as it does on streptomycin-susceptible strains of tubercle bacilli.

SYNERGISM

In vitro experiments by Vennesland and others have shown that PAS when combined with streptomycin exerts a synergistic effect on the tubercle bacillus *in vitro* but this was not discernible to Swedberg or McClosky. Here again, differences in technique, concentrations of the agent, or strain of the bacilli studied, and the amount of inoculum and other technical factors may account for some of the differences, but differences in the products used, as with Duca, might account for some of the others.

Compounds of streptomycin and para-aminosalicylic acid have been reported as effective, but their exact value is still to be determined.

On the other hand, it has been reported that the simultaneous administration of PAS and streptomycin greatly delays the development of resistance to the

latter agent. Arany, Dunner, and others have made this plausible observation and it is being verified on a large scale at the present time in the Veterans Administration hospitals. More work is needed to determine the exact dosage and the time relationship involved in the delay of the development of resistance to streptomycin by the concurrent administration of PAS.

TOXICITY IN MAN

PAS has been administered to many hundreds of human patients with tuberculosis in the past four years. Much of the difference in the results obtained may be ascribed to differences in the toxicity of the material and the dosage used. Lehmann and others have stated that there are no salicylate effects, that is, the patients do not show sweating, tinnitus, or deafness, and that PAS does not exert a direct antipyretic effect, although this latter point does not appear to have been determined by actual experiment. Exceptional instances of slight parenchymal liver damages, as shown by Takata Ara and hippuric acid tests, and slight renal irritation with crystalluria have been reported by a few workers. The chief toxic effects noted in man have been gastrointestinal disturbances with nausea, vomiting, and especially diarrhea. This has been noted particularly early in the course of treatment and may subside thereafter and has been more prominent with tablets than with powder or liquid preparations and much more common with certain preparations than with others, being increased with larger doses of the same preparation. Some materials are usable in large quantities without producing such symptoms while others did cause trouble in very small concentrations. It has also been stated by several workers that the adminstration of PAS increases the susceptibility to toxic effects from salicylates such as acetylsalicylic acid (aspirin). Most writers assume that the gastrointestinal disturbances are a necessary consequence of the treatment and some even report loss of weight and other resultant changes as being of minor importance and not interfering with continuation of the treatment, but others ascribe them chiefly to impurities in the preparation and imply that the benefits from PAS might be secured without incurring these disadvantages.

Doses as low as 8.0 Gm. a day have been reported by some workers as producing toxic effects while others have given more than three times that much without complaint. Lehmann himself recommended from 10 to 15 Gm. per day and most workers appear to have been satisfied with such dosage, but several have not been able to give this dosage and some have advocated considerable increase. PAS has also been administered in 3 per cent to 10 per cent aqueous or in 5 per cent glucose solutions locally and intrapleurally.

CLINICAL RESULTS

Series of human cases treated for tuberculosis have varied from the original two instances reported by Lehmann to 378 cases reported by Vallentin and his associates and there is an even larger series now under treatment in the Veterans Administration. Most writers report marked early symptomatic improvement following the use of PAS. This is usually characterized by a tonic effect with

improved general condition, increase in appetite, improved tissue turgor, less cough and amount of sputum, slowing of the pulse, and respiration with increase in vital capacity and increase in hemoglobin. A drop in temperature has been noted by many writers, particularly in patients who were running high fevers, due to tuberculous pneumonia or pleurisy when treatment started. This drop may be partial or complete, may occur abruptly or gradually and at times may be temporary and followed by a rise some weeks later. The temperature drop is not due to a salicylate effect, according to Lehmann and Sievers, and they imply that it would not occur in patients in whom the fever was not directly a consequence of tuberculosis. Methods of avoiding toxicity so far tried include enteric coating of tablets or granules, applying locally (in aqueous, not fatty, ointment form) intravenous administration and neutralization or administration as an effervescent drink with sodium bicarbonate, sugar, and flavoring agents.

More than 100 patients have been treated with para-aminosalicylic acid at Olive View during the past year for a course of a month or more, in the absence of concurrent administration of streptomycin. These are suitable for an evaluation of the therapeutic, as well as the toxic, effects of PAS. Although all had far advanced pulmonary tuberculosis, many also had visible laryngeal or other local lesions suitable for such evaluation. The chief indication for treatment of these patients was a visible tuberculous lesion such as tuberculosis laryngitis, tracheobronchitis, draining sinus, tuberculosis adenitis, or else a fresh exudative pulmonary lesion such as "spreads" following hemorrhages, operations, et cetera.

Twenty-two of the courses were started in January; 25 in February; 19 in March; and the others in April or May, 1949. The majority received three or four heaping teaspoonfuls or a half medicine glass daily of a powder consisting of PAS mixed with half its weight of sodium bicarbonate and half its weight of sugar, thus containing about 3.0 to 4.0 Gm. of PAS per dose, or 12 to 16 Gm. daily. Others received many capsules or tablets of 0.3 or 0.5 apiece, totaling 5.0 to 10 Gm. daily. Granules, plain, effervescent or enteric-coated, were also tried. Ten patients received daily intravenous injections of 300 cc. of 3.3 per cent PAS in 5 per cent glucose solution.

Nearly half of these patients had previously had some treatment with streptomycin. In more than 20 cases, PAS was given to patients whose organisms had already been found resistant to streptomycin. A number have been given streptomycin after the discontinuance of PAS. In several instances, patients who failed to respond to the PAS showed benefit from later administration of streptomycin.

Our first interest was in toxicity from PAS. The 0.3 Gm. tablets in the lot received originally produced considerable gastrointestinal irritation, diarrhea developing in most of the patients who received more than 6.0 Gm. daily in this form. When the tablets were ground to a powder and administered with sodium bicarbonate and sugar, the irritation was somewhat lessened and doses up to 12 Gm. a day could be administered. Some lots of powder produced similar gastrointestinal irritations and many patients complained of the taste, as well as the nausea and diarrhea from these batches. However, other lots of the powder have

been much better tolerated. In a number of cases, diarrhea developed during the first week and later subsided. In others, diarrhea was not noted until after one or two weeks of treatment and it persisted as long as the offending material was administered but ceased when the same dosage was continued from another lot of PAS.

In at least 3 patients there was noted temporary rise of temperature on the days when PAS was given. In one patient given both PAS and streptomycin, a leukopenia (3,000 leukocytes per cu.mm.) was noted. Several reports were received of PAS tablets being found in the stool, especially the enteric-coated tablets, and in patients with diarrhea such as often developed under the treatment.

Reports of prolongation of prothrombin time or interference with the blood clotting mechanisms, or of secondary avitaminosis B or C following the use of PAS could not be confirmed in our experience.

The majority of the patients who were given PAS described a tonic effect or general feeling of improvement while taking the agent. This was confirmed in about one-half of the cases by a drop in temperature if fever was present, a lessening of cough and of volume of expectoration, and thinning of sputum secretion, improvement in appetite, and gain of weight, and in a number of cases by lessening of gastrointestinal symptoms previously ascribed to tuberculous enteritis. It is too early to report radiologic changes, or changes in bacillary content of the sputum, although both have been observed in individual instances. Several striking instances of improvement in laryngeal ulcers and other visible lesions have also been noted, but this has not been as rapid or as consistently observed as with streptomycin.

A larger number of patients have now been treated at Olive View with streptomycin together with PAS for only one or two days each week. The early clinical response with this regimen has been generally similar to that with streptomycin alone. It is hoped, however, that by this combination the development of streptomycin-resistant tubercle bacilli may be delayed or averted and the patient profit from longer or repeated courses of chemotherapy.

SUMMARY

Both *in vitro* and *in vivo* laboratory tests, as well as extensive clinical investigations, indicate that PAS is a valuable addition to our therapeutic armamentarium for the treatment of tuberculosis.

Most preparations of PAS so far obtainable are insufficiently pure. If the nauseous and noxious contaminants and degradation products could be eliminated, much of the present difficulties in administering the large dosage of PAS that is essential to its successful use might be obviated. Even with the materials now available, gratifying clinical effectiveness may be demonstrated. Combination of PAS with intermittent treatment with streptomycin greatly increases the applicability and effectiveness of chemotherapy in tuberculosis.

SUMARIO

El Acido Para-Aminosalicílico en el Tratamiento de la Tuberculosis

Los ensayos de laboratorio, tanto *in vitro* cuanto *in vivo*, así como extensas investigaciones clínicas, indican que el PAS constituye una valiosa adición a nuestro arsenal terapéutico contra la tuberculosis.

La mayor parte de las preparaciones de PAS obtenibles hasta ahora no son suficientemente puras. Si pudieran eliminarse los contaminantes nauseosos y nocivos y los productos de degradación, se eliminarían muchas de las dificultades con que se tropieza al administrar las altas dosis de PAS que son esenciales para el éxito. Aun con las sustancias hoy día disponibles, puede observarse una eficacia clínica halagadora. La combinación del PAS con la estreptomicinoterapia intermitente acrecienta considerablemente la aplicabilidad y efectividad de la quimioterapia en la tuberculosis.

Acknowledgment

The writer wishes to express his thanks for assistance in obtaining samples of PAS to Doctors W. Feldman and H. Corwin Hinshaw of Mayo Clinic, E. A. Sharp of Parke, Davis & Company, F. Feldmann of the Public Health Service, E. Barrett of Los Angeles, A. Gierson of the Los Angeles General Hospital, R. Smart of Los Angeles, S. Netzer and D. Salkin of the San Fernando Veterans Hospital, Captain E. M. Ricen and Lt. Commander N. Furtos of Corona Veterans Hospital, L. E. Detrick and Mr. Paul P. Spickard of Rexall, Dr. E. G. Upjohn of the Upjohn Co., Dr. J. P. M. Black of Olive View, and to Messrs. W. A. Walker and Brennan of Calco, Mr. H. Silas of Silas and Co., Dr. G. K. Ridge of Ventura, California, and Dr. L. A. Cameron of Los Angeles, and to Mr. J. Blaine of the Delta Laboratory, and Mr. J. Stockton of Hyland Laboratories, Los Angeles.

BIBLIOGRAPHY

(1) Anonymous: Chemioterapia elettiva del B. di Koch Jorgen Lehmann i il suo PAS, Gior. ital. del tubercolosi, Milan, 1948: *2/1*, 45.
(2) Anonymous: PAS in tuberculosis, Am. Prof. Pharmacist, 1947, *13*, 1100.
(3) Anonymous: Three drugs against tuberculosis, Lancet, December 27, 1947, p. 950.
(4) Anonymous: German patents #48491 and #50835. Friedlander, 1887, *2*, 139.
(5) Albores, J. M.: Acido para-aminosalicilico, neuvo agente quimioterapico de la tuberculosis, Semana méd., July 21, 1949, *56*, 136.
(6) Alin, K., and Difs, H.: Clinical experiences with PAS in pulmonary tuberculosis: 1. Therapeutic experiments with PAS: 2. Absorption and excretion of PAS, Nord. med., January 10, 1947, *33*, 151.
(7) Alin, K., and Hellander, S.: Verteilung von PAS in den Geweben, Acta Tuberc. Scandinav., 1948, *22*, 283.
(8) Arany, L. S.: Therapeutic impressions of combined and PAS regimen, Minutes of the Sixth Streptomycin Conference, October 22, 1948, St. Paul, Minnesota, Veterans Administration Branch Office No. 8, Tuberculosis Section, St. Paul, Minn., p. 201.
(9) Barclay, W. R.: The intravenous administration of PAS, Am. Rev. Tuberc., 1949, *60*, 385.
(10) Bardesono, G.: L'acido paraminosalicilico nella tuberculosi polmonare, Minerva med., January 27, 1949, *40*, 131.
(11) Beilstein, F. K.: Handbuch der Organischen Chemie, Berlin, 1918.

(12) BERNHEIM, F.: The oxidation of benzoic acid and related substances by certain mycobacteria, J. Biol. Chem., 1942, *143*, 383.

(13) BERNHEIM, F.: Effect of various substances on oxygen uptake of tubercle bacilli, Science, 1941, *92*, 204; J. Bact., 1941, *41*, 387.

(14) BLOCH, H., LEHR, H., AND ERLENMEYER, H.: Metabolism of tubercle bacilli, Helvet. chim. acta, 1945, *28*, 1406.

(15) BLOCH, R. G., VENNESLAND, K., EBERT, R. H., AND GOMORI, G.: The effect of streptomycin, PAS and their combination on the tubercle bacillus *in vitro* and *in vivo*, Am. Rev. Tuberc., 1949, *59*, 554.

(16) BOGEN, E.: PAS in the treatment of tuberculosis, Ann. West. Med. & Surg., 1949, *3*, 331.

(17) BOGGIANI, B.: L'acide paramino salicilico nella cura della tuberculosi, Policlinico, February 14, 1949, *56*, 193.

(18) BRATTON, A. C., AND MARSHALL, E. K.: A new coupling component for sulfonilamide determination, J. Biol. Chem., 1939, *128*, 537.

(19) BRAY, H. G., RYMAN, B. E., AND THORPE, W. V.: Metabolism of amino hydroxybenzoic acids, Nature, 1948, *162*, 64 (C.A. 42: 8306d).

(20) BRAY, H. G., RYMAN, B. E., AND THORPE, W. V.: Fate of certain organic acids and amides in the rabbit, Biochem. J., 1947, *41*, 212.

(21) British Medical Association Annual Conference, Brit. M. J., July 17, 1948, *2*, 147.

(22) BUCHER, O.: Zur cytotoxischer wirkung von Streptomycin p-amino-salicylsäure und sulfon "Cilag". Streptomycin und Tuberkulose by Fanconi, G., and Loffler, W., Basel, 1948, p. 35.

(23) CAPRETTI, G., INVERNIZZI, M., *et al.*: Richerche cliniche e sperimentali sull'azione dell'acido para-aminosalicilico nella tuberculosi polmonare ed extrapolmonare, Gior. clin. med., 1949, *30*, 405.

(24) CARR, D. T.: Progress in research on PAS. Bull. Nat. Tuberc. A., 1948, p. 157.

(25) CARSTENSEN, B.: PAS behandlung bei darmtuberkulose: Discussions by Westergren, Hedval, Carstensen, Lehmann, Tornell, Hedvall, Lingquist, (photostat) 22 cases.

(26) CARSTENSEN, B., AND SJOLIN, S.: PAS vid lungtuberkulos med sekundar darmtuberkulos, Svenska lakartidningen, April 16, 1948, *45*, 729 (TB. Index, *3*, 488).

(27) CARSTENSEN, B., AND SODERHELM, L.: Miliary tuberculosis treated with PAS and streptomycin, Nord. med., November 5, 1948, *40*, 2039.

(28) CARVALHO, CAMPOS, J. DE: Contribucao para o estude da tuberculose o acide para-amino-salicilico no tratamento da tuberculose pulmonar, J. méd., Pôrto, February 12, 1949, *13*, 177.

(29) CATTANEO, C., AND MORELLINI, M.: Sulle propriete antitubercolari dell'acido p-aminosalicilico contributo sperimentale, Farmaco Pavia, July–August 1948, *3*, 492.

(30) CILAG, LTD.: Summary of the literature on the treatment of tuberculosis with PAS (mimeographed), Schaffhouse, Switzerland.

(31) CORACH, L.: Estado actual del tratamiento de la tuberculosis con el para-aminosalicilico, Prensa méd. argent., March 25, 1949, *36*, 542.

(32) CURCI, O.: Vantaggi e svantaggi della terapia congli antibiotici antitubercolari streptomicina e PAS, Diagnosi Nap., 1949, *5*, 125.

(33) DAVIS, H.: PAS Monthly Bulletin of the Ministry of Health, 1948, *7*, 109 (TB. Index, *3*, 460).

(34) DELAUDE, A., KARLSON, A. G., CARR, D. T., FELDMAN, W. H., AND PFUETZE, K.: Increase in resistance of tuberculosis to NaPAS, Proc. Staff Meet., Mayo Clin., June 22, 1949, *24*, 341.

(35) DEMPSEY, T. G., AND LOGG, M. H.: PAS in tuberculosis: Early results of clinical trials, Lancet, December 13, 1947, *253*, 871.

(36) Desbordes, J., and Henry, J.: Metabolism of PAS in man and in animals, Ann. Pharm. Franc, 1948, *6*, 98 (C.A. 42: 7815i).

(37) Desbordes, J., and Paraf, M.: Naissance d'un nouvel antibiotique entierement synthetique actif contre le bacile de Koch: mode d'action de l'acide para amino salicylique dans la tuberculose, Semaine d. hôp. Paris, January 26, 1949, *25*, 296.

(38) D'Esopo, N.: Discussion: Streptomycin and PAS on resistance, Minutes of the Seventh Streptomycin Conference, April 21, 1949, Denver, Colorado, Veterans Administration Area Office, Tuberculosis Section.

(39) Dickenson, H. G., and Kelly, W.: Estimation of para-amino-salicylic acid in blood and urine, Lancet, February 26, 1949, *1*, 349.

(40) Diczfalusy, E.: Action of salicylic acid derivatives: 1. Investigations on the action of 4-amino-salicylic acid, Arkiv. Kemi, Mineral, Geol., 1947, 24B (no. 13) 1 (C.A. 42: 5506g).

(41) Donaldson, R. I. L.: Clinical study of the use of PAS in the treatment of tuberculosis, Edinburgh M. J., 1949, *56*, 245.

(42) Drain: Absorption spectrum of PAS, J. Chem. Soc., 1949, 1498.

(43) Duca, C. J., Williams, R. D., and Scudi, J. V.: Chemotherapy of tuberculosis: III. *In vitro* and *in vivo* activities of various compounds, Proc. Soc. Exper. Biol. & Med., 1949, *67*, 159 (C.A. 42: 4272d).

(44) Dunner, E.: Effect of combining streptomycin and PAS on resistance, Minutes of the Seventh Streptomycin Conference, April 21, 1949, Denver, Colorado, Veterans Administration Area Office, Tuberculosis Section.

(45) Dye, W. E.: Intravenous PAS: A case report, Eighth Streptomycin Conference, Veterans Administration, Atlanta, Ga., November 10, 1949.

(46) Dye, W. E.: PAS blood levels on different regimens, Minutes of the Seventh Streptomycin Conference, April 21, 1949, Denver, Colorado, Veterans Administration Area Office, Tuberculosis Section.

(47) Eadie, G. S., Bernheim, F., and Fitzgerald, R. J.: The kinetics of the oxidation of benzoic acid by certain mycobacteria, J. Biol. Chem., 1948, *176*, 857.

(48) Eastlake, C., and Barach, A. L.: Use of PAS in chronic pulmonary tuberculosis, Dis. of Chest, July 1949, *16*, 1.

(49) Erdei, A.: PAS in tuberculosis, Lancet, July 10, 1948, *2*, 118; July 17, 1948, *2*, 119.

(50) Erdei, A., and Snell, W.: Pulmonary tuberculosis treated with PAS, Lancet, May 22, 1948, *1*, 791 (TB. Index 3: 488).

(51) Erlenmeyer, H., Prijs, B., Sorkin, E., and Suter, E.: PAS and components of similar structure, Helvet. chim. acta, 1948, *31*, 988 (C.A. 42: 8245d).

(52) Erlenmeyer, H., Sorkin, E., and Vogtli, W.: Characterization of tuberculostatically active amines, Helvet. chim. acta., 1949, *32*, 605.

(53) Erlenmeyer, H., Sorkin, E., and Vogtli, W.: Characterization of tuberculostatically active amines, II, Helvet. chim. acta., 1949, *32*, 1275.

(54) Feldman, W. H., Karlson, A. G., Carr, D. T. and Hinshaw, H. C.: Parenteral administration of PAS in experimental tuberculosis, Proc. Staff Meet., Mayo Clin., April 27, 1949, *24*, 220.

(55) Feldman, W. H., Karlson, A. G., and Hinshaw, H. C.: PAS in experimental tuberculosis in guinea pigs, Proc. Staff Meet., Mayo Clin., October 15, 1947, *22*, 473.

(56) Fitzgerald, R. J., and Bernheim, F.: The effect of salicylic acid on the growth, morphology and virulence of *M. Tuberculosis*, Am. Rev. Tuberc., 1948, *58*, 210.

(57) Fleisch, A. O.: Influence de la Streptomycine et de l'acide p-amino-salicylique sur la respiration cellulaire. In "Streptomycin und Tuberkulose", by G. Fanconi and W. Loffler, Basel, 1948, p. 25.

(58) Forsgren, E.: Hur verkar PAS vid behandling av tuberkulos Svenska Lakartidningen, 1948, *45*, 1834 (Excerpta Medica Tuberculosis, *2*, 89).

(59) Ghielmetti, G.: Preparation of PAS, Farm sci. e tic., 1948, *3*, 51. (C.A. 42: 4553h).

(60) GILLIARD, A.: Note statistique sur le traitment de l'empyeme tuberculeux par l'acide p-amino-salicylique (PAS-Cilag), Schweiz. med. Wchnschr., July 9, 1949, *79*, 625.

(61) GOLDSTEIN, A.: Antibacterial chemotherapy, New England J. Med., January 20, January 27, February 3, 1949, *240*, 98, 117, 180.

(62) GOODACRE, C. L., MITCHELL, B. W., AND SEYMOUR, D. E.: II. The *in vitro* tuberculostatic behaviour of PAS and related compounds, Quart. J. Pharm. & Pharmacol., July–September 1948, *21*, 301, 410.

(63) GRAESSLE, O. E., AND PIETROWSKI, J. J.: The *in vitro* effect of PAS in preventing acquired resistance to streptomycin by *Mycobacterium tuberculosis*, J. Bact., April 1949, *54*, 459.
Also in H. M. Riggins and C. H. Hinshaw's, "Streptomycin and Dihydrostreptomycin in Tuberculosis," 1949, p. 527.

(64) GRILLO, J., AND KRAKAUER, E.: Sobre la quimioterapia de la Mycobacilosis, An. Soc. biol. Bogotá, 1948, *3*, 106.

(65) GULINOV, V. G.: Preparation of aminosalicylic acid from nitrosalicylic acid, J. Chem. Ind., Moscow, 1927, *4*, 909; 1928, *5*, 225 (C. A. 22: 3648) and 1928, *5*, 1019 (C.A. 23: 1632).

(66) HABERLI, E.: Ueber den Nachweis von p-aminosalicylicylsaure in injekttionslösungen, Helvet. pharm. acta, August 28, 1948, *23*, 229.

(67) HART, P. D.: Chemotherapeutic measures in tuberculosis: General assessment and current problems, Am. Rev. Tuberc., 1949, *59*, 223.

(68) HEILBRON, O.: A Dictionary of Organic Compounds, New York, 1938.

(69) HERNER, B.: Effects of benzoic and salicylic acid, Acta physiol. Scandinav., 1944, *8*, Supp., 23, 99.

(70) Herts Pharmaceutical Ltd., Wedwyn, Garden City, Herts "Paramisal" 176 M.I. (photostat) April 1948.

(71) HIRT, R., AND HURNI, H.: The tuberculostatic action of PAS, derivatives and allied substances, Helvet. chim. acta, 1949, *32*: 378.

(72) HOBBY, G. L.: Effect of PAS salt of streptomycin on experimental tuberculosis in guinea pigs, Minutes of the Seventh Streptomycin Conference, April 23, 1949, Denver Colorado, Veterans Administration Area Office, Tuberculosis Section.

(73) HOBBY, G. L.: PAS salt of streptomycin, Eighth Streptomycin Conference, Veterans Administration, Atlanta, Georgia, November 10, 1949.

(74) HODGMAN, C. D.: Handbook of Chemistry and Physics, 18th ed., 1933, pp. 123, 187, 200, 220.

(75) HODGMAN, C. D.: Handbook of Chemistry and Physics, 30th ed., 1948.

(76) HORNE, N. W.: PAS in pulmonary tuberculosis, Edinburgh M. J., June 1949, *56*, 329.

(77) HUG, R., MOESCHLIN, S., AND TANNER, E.: Die Therapie der tuberkulose mit PAS mit besonderer berücksichtigung der bronchial und nieren tuberkulose, Schweiz. med. Wchnschr., April 23, 1949, *79*, 353.

(78) HURNI, H.: Ueber die quantitative Verhälnisse beim Antagonismus zwischen PAS und PABA, Schweiz. Ztschr. f. Path. u. Bakt., 1949, *12*, 282.

(79) HURNI, H.: Is there fastness to PAS? Experientia, 1949, *5*, 128.

(80) IVANOVICS, G.: Antagonism between effects of PAS and salicylic acid growth on *M. tuberculosis*, Proc. Soc. Exper. Biol. & Med., 1949, *70*, 462.

(81) JOULES, F. E., AND NASSAY, E.: PAS acid: A clinical trial in ten patients with pulmonary tuberculosis, Tubercle, 1949, *30*, 98.

(82) JUSTONI, R.: PAS, Farm sci. e tic (Pavia), 1948, *3*, 183.

(83) KARLSON, A. G., AND FELDMAN, W. H.: The effect of combined therapy with streptomycin and PAS on experimental tuberculosis in guinea pigs, Proc. Staff Meet., Mayo Clin., September 28, 1949, *24*, 510.

(84) KARLSON, A. G., AND PFUETZE, K. H.: The effect of combined therapy with strepto-

mycin, PAS and promin on the emergence of streptomycin-resistant strains of tubercle bacilli, Proc. Staff Meet., Mayo Clin., February 16, 1949, *24*, 85.

(85) KEENAN, G. L.: Microscopic identification of acetyl salicylic acid and salicylic acid, The Chemist Analyst, June 1949, *38*, 31.

(86) KLYNE, W., AND NEWHOUSE, J. P.: PAS determination in blood and cerebrospinal fluid, Lancet, October 16, 1948, *2*, 611.

(87) LANGE, N. A.: Handbook of Chemistry, Sandusky, Ohio, 1937.

(88) LEHMANN, J.: Chemotherapy of tuberculosis: The bacteriostatic action of PAS and closely related compounds upon the tubercle bacillus, together with animal experiments and clinical trials with PAS, Svenska Lakartidningen, August 16, 1946, *43*, 2029; also Nord. med., January 10, 1947, *33*, 140 (C.A. 41: 1334g and 1335c).

(89) LEHMANN, J.: PAS, Minutes of the Seventh Streptomycin Conference, April 23, 1949, Denver, Colorado, Veterans Administration Area Office, Tuberculosis Section.

(90) LEHMANN, J.: Personal Communication.

(91) LEHMANN, J. (a) PAS in the treatment of tuberculosis; (b) Determination of the pathogenicity of tubercle bacilli by means of their intermediate metabolism, Lancet, January 5, 1946, *250*, 14.

(92) LEHMANN, J.: Investigations on *Mycobacterium tuberculosis*, Review Gen. Sci., 1947, *54*, 222.

(93) LEMMING, R.: Skin tuberculosis treated with PAS, Lancet, January 1, 1949, *1*, 200.

(94) LENNARTZ, TH.: Antituberculosis compounds: Antibiotic properties of derivatives of orcinol, Z. Naturforsch, 1947, No. 26, 7 (C.A. 42: 8883b).

(95) LEVADITI, C., GIRARD, A., AND VAISMAN, A.: Etude experimentelle des effets antituberculeux de l'acide 4-aminosalicylique, Bull. Acad. nat. med., March 23, 1948, *132*, 210 (TB. Index *3*, 473).

(96) LEVADITI, C., GIRARD, A., *et al.*: Nouveaux essais de chimioterapie experimentelle antituberculeuse par l'acide para amino salicylique, Bull. Acad. nat. méd., May 3, 1949, *133*, 356.

(97) LOEBEL, R. O., SHORR, E., AND RICHARDSON, H. B.: The influence of foodstuffs upon the respiration, metabolism and growth of human tubercle bacilli, J. Bact., 1933, *26*, 139.

(98) LOEBEL, R. O., SHORR, E., AND RICHARDSON, H. B.: The respiratory metabolism of the tubercle bacillus, Nat. Tubere. A. Tr., 1930, *26*, 196.

(99) MCANALLY, D., AND SEYMOUR, D. E.: Pharmacy of sodium PAS, Lancet, *254*, 303 (C.A. 42: 4310h).

(100) MCCLOSKY, W. T., SMITH, M. I., AND FRIAS, J. E. G.: Action of PAS in experimental tuberculosis, J. Pharmacol. & Exper. Therap., 1948, *92*, 447, also in Federation Proc., *7*, 244 (abstract).

(101) MCKEE, C. M., RAKE, G., DONOVICK, R., AND JAMBOR, W. P.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural synthetic origin: I, II, and III, Am. Rev. Tuberc., July 1949, *60*, 90, 109, 121.

(102) MARDIS, R. E.: Fifty cases of pulmonary tuberculosis treated with streptomycin q. 3d and daily PAS for 120d. (Read by title at Eighth Streptomycin Conference, U. S. Veterans Administration, Atlanta, Georgia, November 10, 1949).

(103) MARSHALL, A. K.: Determination of PAS in blood, Proc. Soc. Exper. Biol. & Med., July–August 1948, *68*, 471.

(104) MARTIN, D. D., SPRING, F. S., DEMPSEY, T. G., GOODACRE, C. L., AND SEYMOUR, D. A.: PAS in the treatment of tuberculosis, Nature, March 20, 1948, *161*, 435.

(105) MAURER, G.: Open treatment of cavity with streptomycin and PAS, Schweiz med. Wchnschr., 1948, *78*, 345.

(106) MERCK & Co.: The Merck Index, 5th Edition, 1940.

(107) MITCHISON, D. A.: The present position of PAS, N.A.P.T. Bull., June 1948, Tb. Index, *11*, 83.

(108) Moeschlin, S., Jaccard, G., and Bosshard, M.: Experimentelle untersuchungen uber die therapie der tuberkulose durch kombination von streptomycin mit PAS oder sulfon N azetat, Experientia, April 15, 1948, *4*, 158.

(109) Moeschlin, S., Jaccard, G., Bosshard, M., and Muehlon, W.: Streptomycin, Sulfone, p-Aminosalicylsäure und ihre kombinierte anwendung bei experimentellen Tuberkulose, in "Streptomycin und Tuberkulose" by G. Fanconi and W. Loffler, Basel, 1948, p. 41.

(110) Moeschlin, S., and Schreiner, W.: Vergleich der Kombinstionsterapie von Streptomycin mit Sulfon oder p-Aminosalicylsäure (PAS) bei der experimentellen Tuberkulose, Schweiz. med. Wchnschr., February 12, 1949, *79*, 117.

(111) Mozer, J. J.: L'acide para-amino-salicylique dans la traitement de la tuberculose pulmonaire, Praxis, June 30, 1949, *38*, 595.

(112) Nagley, M. N., and Legg, M. H.: PAS in pulmonary tuberculosis, Lancet, May 28, 1949, *1*, 913.

(113) Neitzel, F.: Condensation products of naphthalone sulfonic acids with aminosalicylic acids (to Durand & Huguenin, S. A.) U. S. 2,048,280 July 21, Ger. 629,953 May 23, 1936 (Cl. 12q: 33) (See Fr. 789,589. C.A. 30: 2019).

(114) Nitti, V., and Curci, O.: L'acido p-aminosalicilico nella terapia della tuberculosi, Progr. med., Napoli, 31, 1949, *5*, 186.

(115) Novy, F. G., and Soule, M. H.: Microbic respiration: Respiration of *M. tuberculosis*, J. Infect. Dis., 1925, *36*, 169.

(116) Oberweger, K. H., Seymour, D. E., and Mitchell, B. W.: Analytical and pharmaceutical studies with PAS, Pharm. J., September 18, 1948, p. 107.

(117) Oberweger, K. H., Seymour, D. E., and Simmonite, D.: I. Analytical and pharmaceutical studies of PAS, Quart. J. Pharm. & Pharmacol., 1948, *21*, 292.

(118) O'Connor, J. A.: Pharmacy of sodium PAS, Lancet, 1948, *254*, 191 (C.A. 42: 4310k).

(119) Olivier, H. R.: Action de l'acide para-amino-salicylique dans le rheumatisme deformant progressif, France méd., 1949, *12*, 6.

(120) Paraf, J., Desbordes, J., Henry, J., and Paraf, M.: Elimination of PAS, Compt. rend. soc. de biol., 1948, *142*, 162 (C.A. 42: 8332g).

(121) Paraf, J., Desbordes, J., and Paraf, M.: L'association streptomycin acide para-aminosalicylique dans le traitement des miliares et des meningites tuberculeuses, Bull. Soc. méd. hôp. Paris, January 21, 1949, *65*, 57.

(122) Paramino Corporation: "Paramycin", 1949.

(123) Pfuetze, K. H., and Pyle, M. M.: Chemotherapy in clinical tuberculosis, Proc. Staff Meet., Mayo Clin., April 27, 1949, *24*, 213.

(124) Pitrie, D.: Preparazione del l'acido p-aminosalicilico medrante reazione di Kolbe, Farmaco Pavia, July-August 1948, *3*, 459.

(125) Poper, A. M.: Catalytic reduction of nitro products, Aniline krasochnaya Prom., 1933, *3*, 391, (C.A. 28: 1671).

(126) Ragas, L.: PAS in the chemotherapy of tuberculosis, Schweiz. med. Wchnschr., April 10, 1948, *78*, 332 (C.A. 42: 4272h) TB. Index *3*, 426.

(127) Ragas, L.: Pharmacology of PAS: Demonstration, distribution in the organism and renal elimination, Schweiz. med. Wchnschr., December 18, 1948, *78*, 1213.

(128) Riggins, H. M.: PAS salt of streptomycin, Eighth Streptomycin Conference, Veterans Administration, Atlanta, Georgia, November 10, 1949.

(129) Riggins, H.M., and Gearhart R.P.: Combined chemotherapy in clinical tuberculosis. (In Riggins, H. M., and Hinshaw, C. H.: "Streptomycin and Dihydrostreptomycin in Tuberculosis", 1949, p. 537).

(130) Rosdahl, K. G.: Some properties and derivatives of PAS, Svensk. kem. Tid., 1948, *60*, 12 (C.A. 42: 3744g).

(131) Rosdahl, K. G.: 4 aminosalicylic acid, U. S. Patent 2,445,242, 1948 (C.A. 42: 7337a).

(132) Rosenberg, K. S.: Slide culture method for tubercle bacilli, Lancet, 1943, *244*, 615.

(133) Saz, A. K., and Bernheim, F.: The effect of benzoic acid and related compounds on the growth of certain mycobacteria. J. Bact., 1942, *44*, 385.

(134) Saz, A. K., Johnston, F. R., Burger, A., and Bernheim, F.: Effect of aromatic iodine compounds on the tubercle bacillus, Am. Rev. Tuberc., 1943, *48*, 40.
(135) Seidel and Bittner: PAS, Ber. d. deutsch. chem. Gesellsch., 1909, *34*, 4553; Monatschr. f. Chem., 1902, *23*, 423.
(136) Sheehan, J. T.: PAS, J. Am. Chem. Soc., *70*, 1665 (C.A. 42: 5439b).
(137) Sievers, O.: Experimental trials with PAS against various kinds of bacteria. (In Swedish) Svenska Lakartidningen, August 16, 1946, *43*, 2047; Nordisk Med., January 10, 1947, *33*, 147.
(138) Simpson, J.: Treatment of tuberculous empyema with PAS, Edinburgh M. J., 1949, *56*, 250.
(139) Sivriere, E.: L'acide para-amino-salicylique dans la traitement de la tuberculose pulmonaire et pleurale, Presse méd., November 13, 1948, *56*, 791.
(140) Soderhjelm, L.: Serum PAS following oral ingestion in children, Texas Rep. Biol. & Med., 1949, *7*, 471.
(141) Solomonides, J., and Bourland, E.: Auto-potentiation of sodium para-aminosalicylate, Foreign Letters, J.A.M.A., June 25, 1949, *140*, 730.
(142) Spring, F. S.: PAS, Edinburgh M. J., 1949, *56*, 237.
(143) Spruiskov, A. A.: Production of aminosalicylic acid, Anilin krasochnaya Prom., 1934, *4*, 21 (C.A. 28: 3724).
(144) Steinlein, H. and Wilhelmi, E.: Chemotherapy of tuberculosis with PAS, Schweiz. med. Wchnschr., December 18, 1948, *78*, 1219.
(145) Steinlein, H.: Die behandlung der tuberkulose mit PAS, Schweiz. Ztschr. Tuberk., 1948, *6*, 378.
(146) Stubbs, A. L. and Williams, R. T.: The oxidation products of orthanilamide in the rabbit, Biochem. J., 1947, *41*, 49.
(147) Suter, E.: Zur chemotherapie der tuberkulose, Schweiz. med. Wchnschr., April 10, 1948, *78*, 324 (TB. Index *3*, 427).
(148) Swedberg, B. and Widstrom, G.: Uber die Zusammenhang zwischen der resistung der T. B. stamme *in vitro* und den chemotherapie effect *in vivo* (photostat).
(149) Swedberg, B., and Widstrom, G.: Treatment of experimental tuberculosis in mice and guinea pigs with PAS and streptomycin, Acta med. Scandinav., 1948, *131*, 116.
(150) Tennent, D. M., and Leland, M. L.: Determination of 4-amino salicylic acid, J. Biol. Chem., 1949, *177*, 873.
(151) Tennant, D. M., and Leland, M. L.: PAS, Federation Proc., 1948, *7*, 195.
(152) Tobie, W. C., and Jones, M. J.: PAS in the metabolism of bacteria, J. Bact., 1949, *57*, 573.
(153) Uhry, P., and Hilfiger, J. P.: A propos des reactions physico chimiques entre l'acide para-amino-salicylique et le bacille de Koch, Bull. Soc. méd. hôp. Paris, April 8, 1949, *65*, 533.
(154) Vallentin, G.: Clinical experiences in the treatment of pulmonary tuberculosis with PAS, Svenska Lakartidningen, August 16, 1946, *43*, 2047; Nord. med., January 10, 1947, *33*, 147.
(155) Vallentin, G., Tornell, E., Beskow, A., Carstensen, B., Thune, R., Helleberg, G., and Lehmann, J.: On the treatment of pulmonary tuberculosis with PAS, Scandinavian Tuberculosis Congress, June 25, 1948.
(156) Venkataraman, A., Venkataraman, P., and Lewis, H.: Metabolism of PAS in the organism of the rabbit, J. Biol. Chem., 1948, *175*, 641 (C.A. 42: 4662g).
(157) Vennesland, K., Ebert, R. H., and Bloch, R. G.: *In vitro* effect of streptomycin and PAS on the growth of tubercle bacilli, Proc. Soc. Exper. Biol. & Med., 1948, *68*, 250 (C.A. 42: 7824h).
(158) Veterans Administration Hospital: Effect of varying PAS dosage in combined PAS, streptomycin therapy, Eighth Streptomycin Conference, Veterans Administration, Atlanta, Georgia, November 10, 1949.
(159) Veterans Administration Hospital: Preliminary reports on PAS Salt of streptomycin,

Eighth Streptomycin Conference, Veterans Administration, Atlanta, Georgia, November 10, 1949.

(160) Veterans Administration Hospital: The nature of a reducing substance in urine during PAS therapy, Eighth Streptomycin Conference, Veterans Administration, Atlanta, Georgia, November 10, 1949.

(161) Veterans Administration Hospital: Enteric coated PAS, Eighth Streptomycin Conference, Veterans Administration, Atlanta, Georgia, November 10, 1949.

(162) Von der Heide, E. C.: Enteric coated PAS, Eighth Streptomycin Conference, Veterans Administration, Atlanta, Georgia, November 10, 1949.

(163) Walker, A.: Mimeographed report of streptomycin study units, Veterans Administration Tuberculosis Division, June 1949.

(164) Warburg, O. H.: Ueber die Stoffwechsel der Tumoren, Berlin, Springer, 1926, 263 pp.

(165) Warner, O. W.: Combined streptomycin and PAS in resistance, Discussion, Minutes of Seventh Streptomycin Conference, April 21, 1949, Denver, Colorado, Veterans Administration Area Office, Tuberculosis Section.

(166) Way, E. L., Smith, P. K., Howie, D. L., Weiss, R., and Swanson, R.: Absorption, distribution, excretion and fate of PAS, J. Pharmacol., & Exper. Therap., 1948, *93*, 368.

(167) Way, E. L., Weiss, R., Howie, D. L., and Smith, R. K.: PAS, Federation Proc., 1948, *7*, 263.

(168) Webb, G. B., Boissevain, C. H., and Ryder, C. T.: Gas requirements of tuberculosis, Am. Rev. Tuberc., 1924, *9*, 534.

(169) Westergren, A., von Rosen, E., Idestrom, G., Hedvall, E., Stavenow, S., Wallgren, A., and Fredholm, T.: Swedish experiences of chemotherapy in tuberculosis, Nord. med., September 13, 1946, *31*, 2111; (Abstracts of World Medicine, August 1947, *2*, 213, Abstract No. 668).

(170) Whittet, T. D.: Pharmacy of sodium PAS, Lancet, 1948, *254*, 268 (C.A. 4310d).

(171) Whittet, T. D.: Preparation of solutions of PAS for use in tuberculosis, Pharm. J., 1947, *159*, 133 (C.A. 42: 2395b).

(172) Whittet, T. D.: Chemist and Druggist, 1947, *147*, 692.

(173) Widstrom, G., and Swedberg, B.: Variationen der Chemoresistenz be einigen Tuberculosis (photostat).

(174) Wilson, B. J., and Armstrong, B. W.: Determination of PAS blood levels, Am. J. Clin. Path., 1949, *19*, 886.

(175) Wyss, O., Rubin, M., and Strandskov, F. B.: Mechanism of sulfonamide action of substituted p-aminobenzoic acids, Proc. Soc. Exper. Biol. & Med., 1943, *53*, 155.

(176) Youmans, A. S., and Youmans, G. P.: The effect of Tween 80 *in vitro* on the bacteriostatic activity of 20 compounds for *Mycobacterium tuberculosis*, J. Bact., 1948, *56*, 245.

(177) Youmans, G. P.: Tuberculostatic action of chloromycetin *in vitro* and *in vivo*, Proc. Soc. Exper. Biol. & Med., 1948, *67*, 426.

(178) Youmans, G. P., Youmans, A. S., and Osborne, R.: Combined effect of streptomycin and PAS on experimental tuberculosis in mice, Journal Lancet, 1947, *67*, 403.

(179) Youmans, G. P.: The effect of PAS *in vitro* and *in vivo* on virulent human type bacilli, Quart. Bull. Northwestern Univ. M. School, 1946, *20*, 420.

(180) Youmans, G. P., and Raleigh, G. W.: The effect of PAS on tuberculosis, Soc. Am. Bact., 1947; Federation Proc., May 5, 1947, p. 65; J. Bact., 1917, *54*, 65.

(181) Youmans, G. P., and Raleigh, G.: The use of mice in experimental chemotherapy of tuberculosis: III. The histologic biologic assay of chemotherapeutic action, J. Infect. Dis., 1948, *82*, 221; 1948, *82*, 205.

(182) Youmans, G. P., Raleigh, G. W., and Youmans, A. S.: The tuberculostatic action of PAS, J. Bact., 1947, *54*, 409.

Case Reports

THROMBOSIS OF CEREBRAL VESSELS WITH NECROSIS OF THE BASAL NUCLEI[1]

A Complication of Tuberculous Meningitis and Streptomycin Therapy

R. H. RIGDON AND EDWARD J. LEFEBER

(Received for publication October 11, 1949)

INTRODUCTION

The symptoms of tuberculous meningitis appear to be due principally to five effects upon the central nervous system: (a) mechanical irritation; (b) hypersensitivity; (c) vascular occlusion; (d) extension of the infection to the nervous system; (e) increased intracranial pressure (1). Acute arteritis with an associated thrombosis is a frequent pathologic finding in this type of meningitis. Focal areas of cerebral necrosis, secondary to these vascular lesions, usually are minimal and without clinical significance. Two cases of tuberculosis with transverse myelitis were recently reported in which the degeneration within the spinal cord was secondary to vascular thrombosis (2).

It would seem that necrosis of brain tissue accompanying tuberculous meningitis is more frequent now than previously. It may be that this complication is associated with the newer types of therapy. Baggenstoss and associates (3) in 1947 reported 5 cases of tuberculosis treated with streptomycin and in 3 there were vascular lesions within the meninges. In the first case "there was extensive involvement of the blood vessels with necrosis of the walls." In the second, "in the right frontal lobe, the midbrain and pons varolii there were small regions of necrosis which were interpreted as infarcts resulting from acute arteritis of the meningeal vessels, although some of them may have been tubercles." In their third case "the blood vessels and the adjacent cortex were involved by direct extension of the inflammatory process. Many of the vessels were occluded by organized thrombi." One of the cases treated with streptomycin and reported by Moffit and associates (4) had considerable cerebral damage as shown by the following: "One case of tuberculous meningitis presented marked auditory and labyrinthrine loss, nystagmus, disordered opthokinetic response and other neurologic signs indicative of widespread central nervous system damage."

Endarteritis is a frequent accompaniment of tuberculous meningitis; however, cerebral necrosis which is secondary to it apparently is minimal. Macgregor and Green (5) carefully studied the brain in 88 cases of tuberculous meningitis and state that "in almost all cases arteries involved in these meningeal deposits showed proliferative endarteritis, which in some instances led to their total occlusion."

Recently we have had an opportunity to study at autopsy two cases of tuberculous meningitis, both of which showed vascular occlusions and large areas of necroses in the basal nuclei.

CASE REPORTS

Case 1 (no. 9035E). A 30-year-old white male who worked as a department store clerk consulted one of us (EJL) on July 1, 1948 for a persistent, severe, suboccipital headache of three

[1] From the Departments of Pathology and Medicine, School of Medicine, University of Texas, Galveston, Texas.

weeks' duration. There had been a diffuse soreness in the region of the upper jaw during the preceding week; however, this had subsided. The headache was present on awakening and throughout the day became progressively more severe. He would awaken about three or four o'clock each morning with this pain. His neck would feel stiff, but after moving it several times the stiffness would disappear. Two aspirin tablets taken each morning, afternoon, and evening gave relief within ten minutes. His head would throb following any physical exertion. Accompanying these headaches was a feeling of fatigue. Chills and fever were noted for the first and only time two days before he consulted a physician.

There was a history of intermittent headaches for the preceding nine years. These would occur about three or four o'clock in the morning and usually were precipitated by a prebedtime lunch of candy, milk, or soda water. He had a chronic postnasal drip for many years. While in the army a few years previously he had an illness characterized by fever and vertigo which persisted for two months. Venereal diseases were denied. The family history was noncontributory.

On examination the temperature was 100.2°F., pulse 106 per minute, and the blood pressure 140 systolic, 88 diastolic in mm. of mercury. The patient did not appear acutely ill and nothing significant was found on physical examination. The urine was negative. A hemogram showed 4.57 million red blood cells per cu. mm., 15 Gm. (97 per cent) hemoglobin per 100 cc., 9,150 white blood cells per cu. mm., with 78 per cent polymorphonuclear leukocytes, 20 per cent lymphocytes, and one per cent each of eosinophils and monocytes. All agglutination tests were negative on two different occasions. Serologic test for syphilis (Kline) was also negative. A roentgenogram of the chest was normal.

Six days after the original visit, the pain returned to the right upper jaw. A low-grade fever (101°F.), according to the patient, continued to be present. He was advised to enter the hospital but refused. His eyes were refracted by an optician and the following day a dentist extracted an upper third molar, the root of which was inflamed but no abscess was present.

Fifteen days after consulting us for the first time, the patient entered the hospital. The temperature was 102°F., pulse 101 per minute, respirations 22 per minute, and the blood pressure was 140 systolic, 94 diastolic. Urinalysis, hemogram, agglutination tests, and roentgenograms of the chest showed no significant changes from the studies made two weeks previously. Nothing was found on physical examination. He continued to complain, however, of a severe headache. Two days following hospitalization "drowsiness" was first noted. At this time he began to note numbness in his hands.

On neurological examination, questionable papilledema of the right disc was found. There was no nuchal rigidity. The cerebrospinal fluid pressure was 420 mm. of water. The

---

PLATE I

FIG. 1. Case 1. The degeneration within the basal nuclei is characterized by gliosis and many "gitter cells." No caseation or tuberculous granulation tissue is present in the area of degeneration. H&P ×125.

FIG. 2. Acid-fast bacilli are present within the caseous areas in the meninges. Ziehl-Neelsen's ×1,425.

FIG. 3. An artery in the meninges with its lumen markedly decreased by fibrous tissue. The necrosis in the basal nuclei is considered to be secondary to this vascular lesion. H&P ×100.

FIG. 4. A narrow zone of cortical degeneration in the temporal lobe adjacent to the tuberculous reaction in the meninges. This too is considered to be secondary to the vascular lesion. H&P ×125.

FIG. 5. This occluded artery in the meninges is surrounded by caseous material in which the acid-fast bacilli were demonstrated. A chronic inflammatory reaction is also present. H&P ×125.

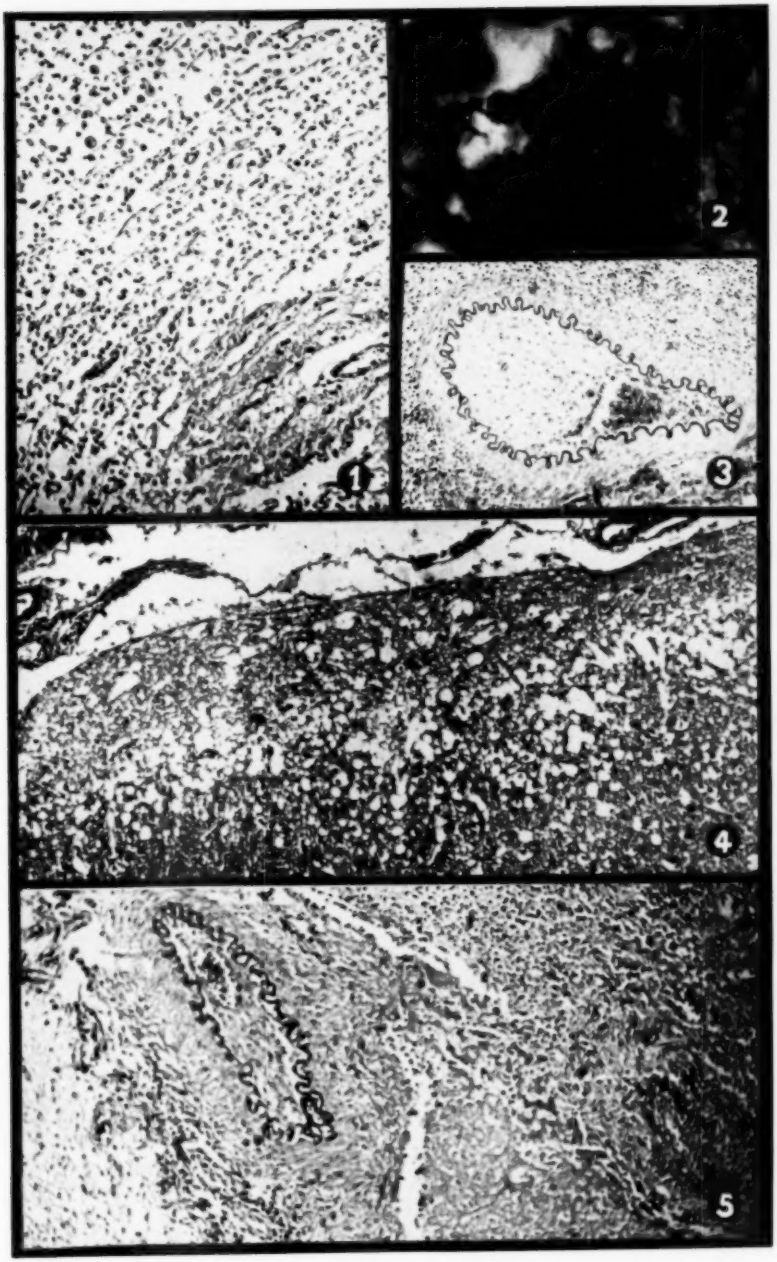


PLATE I

fluid was slightly cloudy and contained 242 cells per cu. mm. with 50 per cent each of polymorphonuclear leukocytes and lymphocytes. There were 168 mg. of protein and 13 mg. of sugar per 100 cc. A gram-positive diplococcus, probably a pneumococcus, was cultured from the cerebrospinal fluid. Penicillin was given intramuscularly and sulfadiazine orally. The following day nuchal rigidity was present, and dizziness and projectile vomiting occurred from time to time. Five days following hospitalization, Cheyne-Stokes respirations were present for the first time. The patient became irrational, and on the eighth hospital day he was semicomatose and his speech was unintelligible. Two days later the right side of the face was weak. This was followed by weakness of the right upper and lower extremities. At this time there was choking of the right disc. Sustained right ankle clonus was noted on the fourteenth day of hospitalization. The cerebrospinal fluid pressure varied between 245 and 550 mm. of water, and the sugar varied between 11 and 32 mg. per 100 cc.

The patient appeared chronically ill on the thirty-second day following hospitalization. At this time he lay quietly in bed and responded occasionally to questions by opening his eyes. Nuchal rigidity was definite. The pupils reacted to light and there were two to three diopters of papilledema which was accompanied by a marked venous engorgement on the right. A slight facial weakness, deviation of the uvula to the left, and weakness of both the right upper and lower extremities were present. He had a loss of voluntary movements on the right, a sustained right ankle clonus, absent abdominal reflexes bilaterally, positive Babinski test bilaterally, with a motor aphasia and incontinence of urine. During the thirty-second and thirty-third days of hospitalization, the patient progressively became comatose and developed Cheyne-Stokes respirations. During this time he had a Jacksonian type of seizure involving the left side of the face, left arm and leg. Following this convulsion there were athetoid movements of the affected arm and leg. The flaccid paralysis on the left was replaced by a spastic type with exaggerated deep reflexes. The left-sided paralysis was followed by spasticity of the extremities on the right. The cerebrospinal fluid was slightly cloudy and the pressure was 330 mm. of water. The protein was 130, sugar 53.7, and the chlorides 585 mg. per 100 cc. There were 140 white blood cells per cu. mm. with 77 per cent lymphocytes. The erythrocyte count was 3.9 million and the hemoglobin was 76 per cent. The white blood cell count was 10,400 per cu. mm. with 67 per cent segmented forms, 6 per cent stabs, 18 per cent lymphocytes, 8 per cent monocytes, and one per cent eosinophils. Urinalysis and a roentgenogram of the skull were normal.

On the thirty-third day of hospitalization the brain was explored by multiple needle insertions, but nothing abnormal was encountered. A ventriculogram showed a slight enlargement of the entire ventricular system with the exception of the third and fourth ventricles. There seemed to be a block between the ventricular system and the spinal cord.

The patient remained in an unconscious vegetative state from the fortieth day until his death on the eighty-second day of hospitalization. During this period there was a remittent type of fever varying between 100° and 102°F. The systolic blood pressure increased during this time from 146 to 200 mm. of mercury and the diastolic from 110 to 138 mm. of mercury.

Specific therapy consisted of the following: (*1*) intrathecal penicillin, 275,000 units; (*2*) intrathecal streptomycin, 400 mg.; (*3*) sulfadiazine, 181 Gm., maintaining a blood level between 6 and 10 mg. per 100 cc.; (*4*) intramuscular streptomycin, 90 Gm.; (*5*) intramuscular and intravenous penicillin, 54,830,000 units. Despite the prolonged and intensive therapy, the patient never regained consciousness. During the last two months of life, tube-feeding was required to maintain his nutrition. He died quietly on the eighty-second hospital day.

Cerebrospinal fluid cultures were negative three times for acid-fast organisms, and blood cultures were sterile on two occasions. During the last month of life the urine showed a moderate amount of sugar (1 to 2 plus) and a large amount of albumin (4 plus). An indwelling catheter was present during this time. The blood sugar was 108 mg. per 100 cc. on the thirty-second day of hospitalization and 81.1 mg. per 100 cc. on the thirty-eighth day. An intradermal tuberculin test (1:10,000) was negative.

Autopsy: The autopsy was performed four hours following death. Only the pertinent

findings are included in this summary. The convolutions of the brain were flattened and the ventricles were dilated. The meninges about the base were thickened. This was most marked between the frontal and temporal lobes. In this area of the meninges microscopic foci of caseous necrosis were present (Plate I, figure 5). Surrounding these areas of necrosis were collections of lymphocytes and more distally there were a few fibroblasts. No giant cells or epithelioid cells were found in any of these sections. Many acid-fast bacilli were demonstrated in the areas of caseation (Plate I, figure 2). The lumen of the small arteries surrounded by the exudate were frequently occluded by a proliferation of their intima (Plate I, figure 3). An acute arteritis was not observed in any of these vessels.

In multiple sections through the brain no tuberculomata were found; however, the anterior portion of both basal nuclei was degenerated (figure 6). "Gitter cells" infiltrated these areas of necrosis (Plate I, figure 1). No caseation, tubercles, giant cells or epithelioid cells were found in these areas of degeneration within the brain tissue. Areas of the cortex

FIG. 6. Case 1. The anterior portion of the basal nuclei shows extensive ischemic necrosis

of the brain adjacent to the Sylvian fissure frequently showed a zone of degeneration (Plate I, figure 4). A few mononuclear cells were present in the dura covering the brain and spinal cord.

The lungs showed an extensive bronchopneumonia with focal abscesses. In the left pleural cavity there were 200 cc. of straw-colored fluid. All the viscera were carefully examined for tuberculosis; however, no lesions were found.

Anatomic diagnosis: Tuberculous meningitis with endarteritis obliterans and bilateral necrosis of basal nuclei; bilateral bronchopneumonia; parenchymatous degeneration of viscera.

Case 2 (no 3.741F). J. M., a 3-year-old Mexican female, was admitted to the hospital with a history of fever, stiff neck, and anorexia. She was well until two weeks before, at which time she became lethargic and a week later complained that her legs "hurt" when she tried to walk. The temperature on admission was 103.6°F., pulse 96, and respiration 18 per minute. The weight was 24 pounds. The child was very irritable. The skin was warm and dry. A large node was present in the right axilla. The cervical and inguinal nodes were small but palpable. The left pupil was slightly larger than the right. Both reacted sluggishly to light. The neck was stiff. All deep reflexes were hypo-active; Babinski was negative and the Kernig's sign positive, bilaterally. Ankle clonus was absent.

Lumbar puncture on admission showed xanthochromic fluid with 39 cells, 37 of which

were lymphocytes and 2 polymorphonuclear leukocytes, sugar 29 mg. per 100 cc., chlorides as sodium chlorides 359 mg. per 100 cc., and proteins, 1,560 mg. per 100 cc. Two days later acid-fast bacilli were demonstrated in the cerebrospinal fluid. A 1:1,000 tuberculin test was 2 plus in 72 hours. Hematologic studies were essentially normal except for an occasional period of leukocytosis, at which time there were some metamyelocytes present. Urine examination was essentially negative except for acetone on admission and a small amount of albumin. Microscopic hematuria was present on one examination. Roentgenograms of the chest showed an increased density in the lower portion of the right hilum and increased bronchovascular markings in the right lower lung.

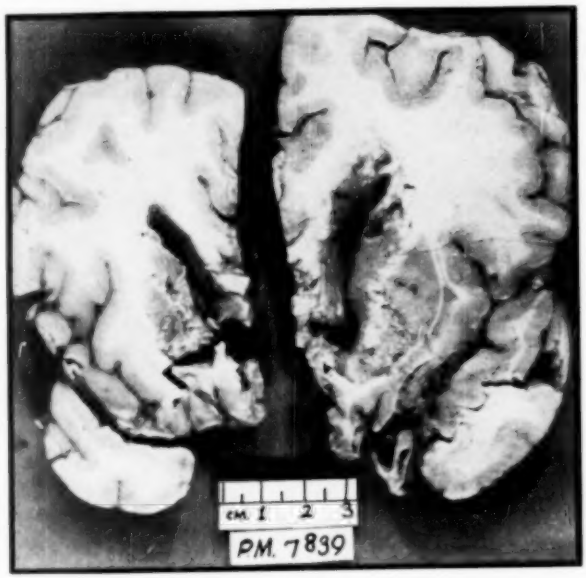


FIG. 7

The child was given a total of 22.1 Gm. of streptomycin during the 55 days of hospitalization; 0.25 Gm. was given intramuscularly usually twice a day and on eight occasions 0.2 Gm. was given intrathecally. Para-aminosalicylic acid was also administered. Little change was observed during the period of hospitalization. During the latter days of life she refused food and water and it was necessary to give her fluids by hypodermoclysis. Death occurred quietly on the fifty-fifth day of hospitalization.

Pathological examination: The autopsy was performed twenty-four hours following death. The body had been embalmed. Only the pertinent data are included in this report.

Several small tuberculous foci were present in the subpleural region of the right upper lobe. These were caseous areas usually well encapsulated. Several caseous lymph nodes were present in the hilar region. One tubercle was found in the spleen. The only other tuberculous lesion was found in the cranial cavity. The brain weighed 1,000 Gm. The convolutions were flat. The entire ventricular system was markedly dilated. A gelatinous exudate covered the brain and was most extensive about the base. The anterior portion of the basal nuclei was soft in focal areas (figure 7). In these the normal architecture was completely replaced by gliosis and gitter cells (Plate II, figure 2). Many capillaries infiltrated these zones of ne-

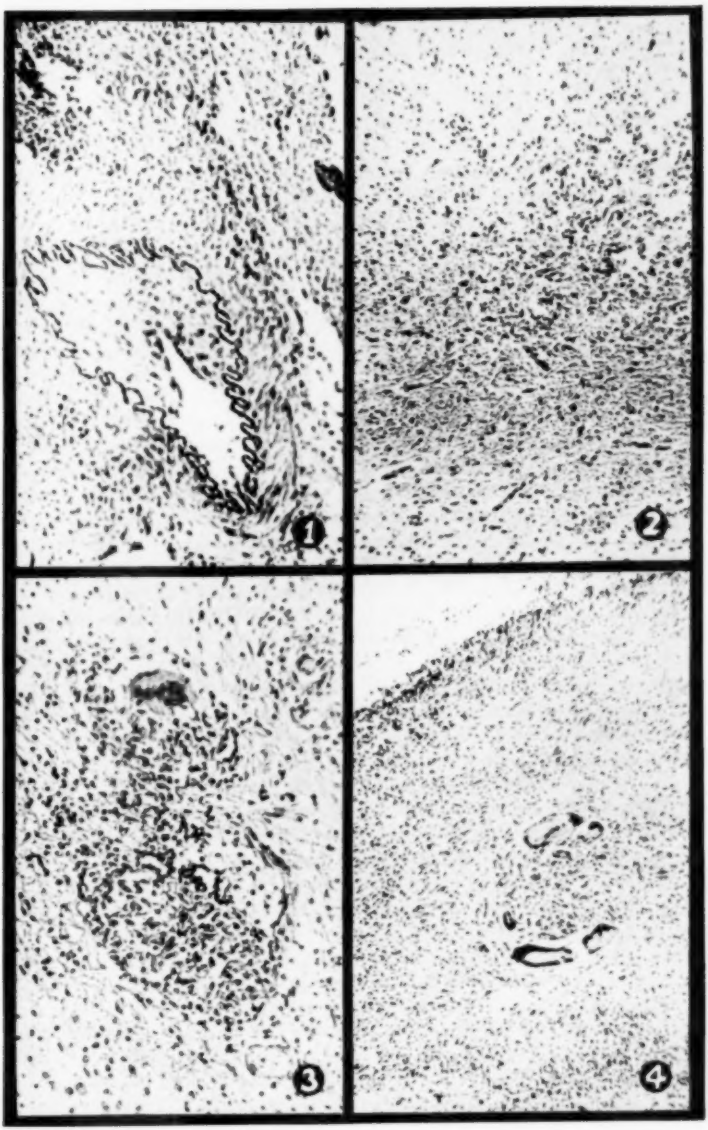


PLATE II

FIG. 1. Case 2. A markedly occluded artery occurs in this area of tuberculous meningitis. H&P ×96.

FIG. 2. The degeneration within the basal nuclei is characterized by extensive gliosis. No caseation is present. H&P ×96.

FIG. 3. A few microscopic tubercles are present within the cortical tissue of the brain. H&P ×330.

FIG. 4. The meningeal reaction is characteristic of tuberculosis. Many tubercles, giant cells, epithelioid cells and lymphocytes are present. H&P ×90.

FIG. 7. Case 2. Degeneration is present within the basal nuclei of this brain. The tuberculous meningitis is widespread in this case.

croses. No caseation, giant cells, or epithelioid cells were found within these areas of degeneration in the basal nuclei. Collections of lymphocytes were present around many of the blood vessels in areas of the cortex of the brain. An occasional tubercle was present in the brain tissue (Plate II, figure 3). A large collection of lymphocytes and a small tubercle-like lesion were found adjacent to the wall of the ventricle in one section. The subarachnoid space was the site of a very extensive reaction characterized by many lymphocytes, giant cells, epithelioid cells, tubercles, and focal areas of caseation (Plate II, figure 1). None of the vessels showed an acute arteritis. No acid-fast bacilli were demonstrated in the pulmonary lesion and in the meninges.

Anatomic diagnosis: Tuberculous foci in the right upper lobe of lung; caseous lymph nodes about the hilum (Gohn lesion); tubercles in the spleen; tuberculous meningitis; hydrocephalus; healed arteritis with fibrous occlusion of lumen of some arteries in meninges; ischemic necrosis of basal nuclei; pulmonary edema; parenchymatous degeneration of viscera.

DISCUSSION

The finding of a gram-positive coccus in the cerebrospinal fluid of Case 1 at first was thought to represent the cause of the infection which was believed to be a brain abscess. The subsequent clinical course, however, made this diagnosis unlikely. It was thought by some that the patient had a tuberculous meningitis although no organisms were demonstrated either in direct smears from the cerebrospinal fluid or in cultures, and the skin reaction to tuberculin was negative. Furthermore, the roentgenograms of the chest did not show any tuberculous foci. The etiology of the vegetative state in which the patient persisted during the last two months of life was not satisfactorily diagnosed clinically.

From the clinical history it is difficult to conclude the exact time of onset of symptoms relative to the meningitis in Case 1. About one month before admission to the hospital the headaches became more severe, and two days following hospitalization "drowsiness" and numbness in the hands were first noted. Approximately ten days later paralysis occurred, involving the right side of the body. These symptoms may have been related to the beginning of the necrosis in the basal nuclei. At this time the patient had not received any streptomycin; therefore, one could not consider the development of coma relative to streptomycin therapy. In view of the pathologic findings, it would appear likely that the vascular occlusions were developing at the time specific therapy was instituted. Therapy apparently inhibited the progression of the lesion and thereby indirectly prolonged the time in which the arterial thrombosis progressed to produce the necrosis in the basal nuclei. Death occurred on the eighty-second and the fifty-fifth days of hospitalization, respectively, in the two cases.

The histologic reaction to the acid-fast bacilli in Case 1 is certainly different to that usually found in the meninges in tuberculous infection. The absence of giant cells and epithelioid cells and the presence of extensive caseation would suggest a hypersensitive type of response. However, the presence of small arteries with a proliferation of their intima without any cellular infiltration of their walls is not the usual vascular lesion observed in a hypersensitive type of tubercular inflammation. The localization of the process within the area of the Sylvian fissures may account for the absence of microorganisms within the spinal fluid and the atypical manifestations as observed in this case.

The tuberculous meningitis in Case 1 is interesting from the standpoint of pathogenesis. No lesions were found in the viscera to suggest either an active or healed tuberculous focus. Rich (1) cites a case in which there was a tuberculous meningitis without any demonstrable visceral lesions. The extent of meningeal involvement in Case 1 was much

less than we usually find at autopsy. The absence of giant cells, epithelioid cells, tubercles, and a diffuse exudative reaction in the meninges made the diagnosis difficult until many acid-fast bacilli were demonstrated.

As tubercle bacilli were never cultured during life or at postmortem and as the skin reaction to tuberculin was negative, it was not possible to establish with certainty that the brain lesions were tuberculous. Nevertheless, the finding of the acid-fast bacilli in the necrotic areas makes it highly probable that the lesions were tuberculous in origin.

The clinical and pathologic findings in Case 2 were typical of a tuberculous infection. The child survived for more than two months after the onset of the meningitis. The histologic reaction in these 2 cases was quite different. Nevertheless, in both there were arteries occluded and areas of ischemic necrosis within the basal nuclei.

The presence of small arteries in the meninges with their lumens occluded and the ischemic type of necrosis in the anterior portion of the basal nuclei, no doubt, were related. The anterior portion of the caudate nucleus is supplied by central branches that arise from the anterior cerebral artery and the anterior communicating artery. Branches from the middle cerebral artery also supply portions of the head of the caudate nucleus. Other cortical branches from the above arteries supply the adjacent portions of the cortex. Occlusion of the latter vessels may account for the necrosis in the cortex of the temporal and frontal lobes of the brain.

It is important to remember that acute arteritis and thrombosis are frequent findings in cases of tuberculous meningitis. If the course of this infection is modified and life is prolonged through the use of streptomycin or any other form of therapy, vascular thrombosis with subsequent necrosis of brain tissue, no doubt, will be a likely complication. The likelihood of damage to the central nervous system, secondary to vascular occlusions, should be remembered when one considers the possible complications of streptomycin other than its specific action on the eighth nerve. Macgregor and Green (5) did not report ischemic necrosis of the basal nuclei in any case in their group of 88 cases of tuberculous meningitis studied before the advent of streptomycin.

A vascular lesion similar to the one observed in the present cases may have occurred in the group discussed by Keefer (6). In this there were 7 cases reported by Hinshaw to the Committee on Chemotherapeutics.

> One died on the fifth day of treatment. . . . The other six showed evidence of clinical improvement. Four of these are still living . . . the outlook with regard to eventual prognosis in these four patients is extremely doubtful. One is blind, one is deaf, one had pronounced bulbar symptoms with dysarthria and there is some question of mental retardation. . . . It is impossible to say how the abnormalities should be interpreted, but Dr. Hinshaw has never known tuberculous meningitis to behave in that manner.

The baby with tuberculous meningitis treated with streptomycin by Cooke and associates (7) developed peculiar neurological signs, many of which had disappeared by the twentieth week. An explanation of the mechanism of these symptoms was not attempted by the authors.

Paine (8) in reporting 32 cases of meningitis treated with streptomycin stated:

> At the time when they were reported, 13 of the group of patients were still living. The incidence of permanent neurologic damage was high, however, and the survivors had been followed for only short periods after the cessation of streptomycin.

A case of tuberculous meningitis reported as recovered by McMahon (9) is interesting in view of the fact that among other symptoms "a transient right-sided partial hemiplegia, involving the arm, leg and trunk, developed." This could be explained readily as the

result of a small area of ischemic necrosis secondary to a vascular occlusion such as we have found in the 2 cases being reported.

From these 2 cases of tuberculous meningitis, it is suggested that prolongation of life through the use of streptomycin may permit sufficient time to elapse for the lumina of some of the small arteries in the meninges to become occluded, resulting in foci of ischemic necrosis. Whether these foci will be found to be more frequent in the basal nuclei must await further study.

SUMMARY

Two cases of tuberculous meningitis are reported in which the lumen of some of the smaller arteries in the meninges was occluded by an endarteritic type of lesion. In both cases there were areas of ischemic necrosis in the basal nuclei which were considered to be secondary to the vascular lesions. Prolongation of life following the onset of tuberculous meningitis through the use of the newer chemotherapeutic agents may permit the acute arteritis frequently found in tuberculous meningitis to progress to complete occlusion, thus producing ischemic necrosis within the brain. This necrosis may be significant from a clinical standpoint.

SUMARIO

Trombosis de los Vasos Cerebrales con Necrosis de los Núcleos Basales: Complicación de la Meningitis Tuberculosa y la Estreptomicinoterapia

En los dos casos de meningitis tuberculosa comunicados, la luz de algunas de las más pequeñas arterias de las meninges estaba ocluída por una lesión de tipo endarterítico. En ambos casos había focos de necrosis isquémica en los núcleos basales que parecían ser secundarios a las lesiones vasculares. La prolongación de la vida después de la iniciación de una meningitis tuberculosa por virtud del empleo de los nuevos agentes quimioterapéuticos puede permitir que la arteritis aguda observada frecuentemente en dicho estado avance a una oclusión total, produciendo necrosis isquémica en el cerebro. Clínicamente, esa necrosis puede ser importante.

Acknowledgment

The photographs were made by Mr. F. W. Schmidt of the Department of Medical Illustrations.

REFERENCES

(1) Rich, A. R.: Pathogenesis of Tuberculosis. Charles C Thomas, Springfield, Ill., 1944.

(2) Rigdon, R. H.: Transverse myelitis accompanying tuberculous meningitis, Am. Rev. Tubere., 1947, *55*, 332.

(3) Baggenstoss, A. H., Feldman, W. H., and Hinshaw, H. C.: Streptomycin in miliary tuberculosis: Its effects on pathological lesions of generalized miliary tuberculosis in human beings, Am. Rev. Tubere., 1947, *55*, 54.

(4) Moffitt, O. P., and Norman, W. B.: Streptomycin toxicity to the labyrinth, Ann. Otol., Rhin. & Laryng., 1948, *57*, 930.

(5) MacGregor, A. R., and Green, C. A.: Tuberculosis of central nervous system, with special reference to tuberculous meningitis, J. Path. & Bact., 1937, *45*, 613.

(6) Keefer, C. S., *et al.*: Streptomycin in the treatment of infections: A report of 1,000 cases, J.A.M.A., 1946, *132*, 4.

(7) Cooke, R. E., Dunphy, D. L., and Blake, F. G.: Streptomycin in tuberculous meningitis: Report of its use in one-year-old infant, Yale J. Biol. & Med., 1946, *18*, 221.

(8) Paine, T. F., and Finland, M.: Use of streptomycin in treatment of meningitis, M. Clin. North America, 1947, *31*, 1092.

(9) McMahon, B. T.: Recovery from tuberculous meningitis: Report of a case, Am. Rev. Tubere., 1926, *13*, 216.

A CASE OF GENERALIZED HEMANGIO-SARCOMATOSIS ERRONEOUSLY CONSIDERED AS GENERALIZED TUBERCULOSIS[1]

MARTHA D. COLLINS[2] AND HYMAN FISHER

(Received for publication June 24, 1949)

The patient whom this report concerns was referred to the Veterans Administration Tuberculosis Hospital at Sunmount, New York, because of blood-streaked sputum, evidence of a pericarditis, pleural effusion, a swelling in the left neck, three sputum smears positive for acid-fast bacilli, and the appearance of a fine mottling in both lung fields suggestive of generalized miliary tuberculosis. He was seriously ill on arrival at the hospital. Roentgenograms revealed a considerable increase in the mottled shadows in both lung fields, and the situation appeared so critical that it was deemed advisable to administer streptomycin without awaiting bacteriologic proof of tuberculosis.

CLINICAL HISTORY

The patient was a 25-year-old married white male who had been a truck driver. A cousin had died from pulmonary tuberculosis, but the patient denied contact with him. During armed service in the South Pacific area he had contracted malaria. Except for relapses of malaria, the patient had been well up to July 15, 1948, at which time he experienced mild chest pain accompanied by an unproductive cough. Two days later, while at work, he developed a sudden severe substernal pain for which he was hospitalized for sixteen days. Blood-streaked sputum had been raised, but roentgenograms of the chest appeared normal.

A short time after discharge from the hospital, the chest pains and blood-streaked sputum had recurred, and on August 18, 1948 he had again been hospitalized. Roentgenograms on August 19 (figure 1) revealed a pleural effusion in the left chest and a transverse enlargement of the cardiac shadow. An electrocardiogram showed inversion of T waves and depression of the S-T segments. Blood smear for malaria parasites, serological tests for brucellosis, and sputum smears for acid-fast organisms were negative. A diagnosis of nonspecific pericarditis was made. Because of fever, penicillin was administered, and after ten days a drug reaction developed that was characterized by angioneurotic edema of the face, generalized urticaria, and arthralgia. Roentgenograms of the chest on September 20 (figure 2) revealed soft, irregular mottling in both lung fields. Following his second hospital discharge, he failed to regain his health. On October 12 stiffness of the neck and a swelling of the left neck occurred, accompanied by headaches and retro-ocular pain. Bloody sputum increased in amount.

On October 26 the patient had been readmitted to the hospital acutely ill. Rales were present in both lung fields. For the first time a loud pulmonic systolic murmur was heard which terminated in an accentuated pulmonic second sound. Roentgenogram of the chest on the same day (figure 3) showed an increase of pulmonary shadows over previous examinations and a further enlargement of the transverse cardiac diameter. An enlarged, somewhat tender lymph node was discovered at the angle of the left jaw. On three occasions, acid-fast bacilli were observed in smears of concentrates of sputum. A diagnosis of tuberculosis was made and the patient was transferred to the Veterans Administration Hospital, Sunmount, New York.

---

[1] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] From Tuberculosis Service, VA Hospital, Tucson, Arizona.

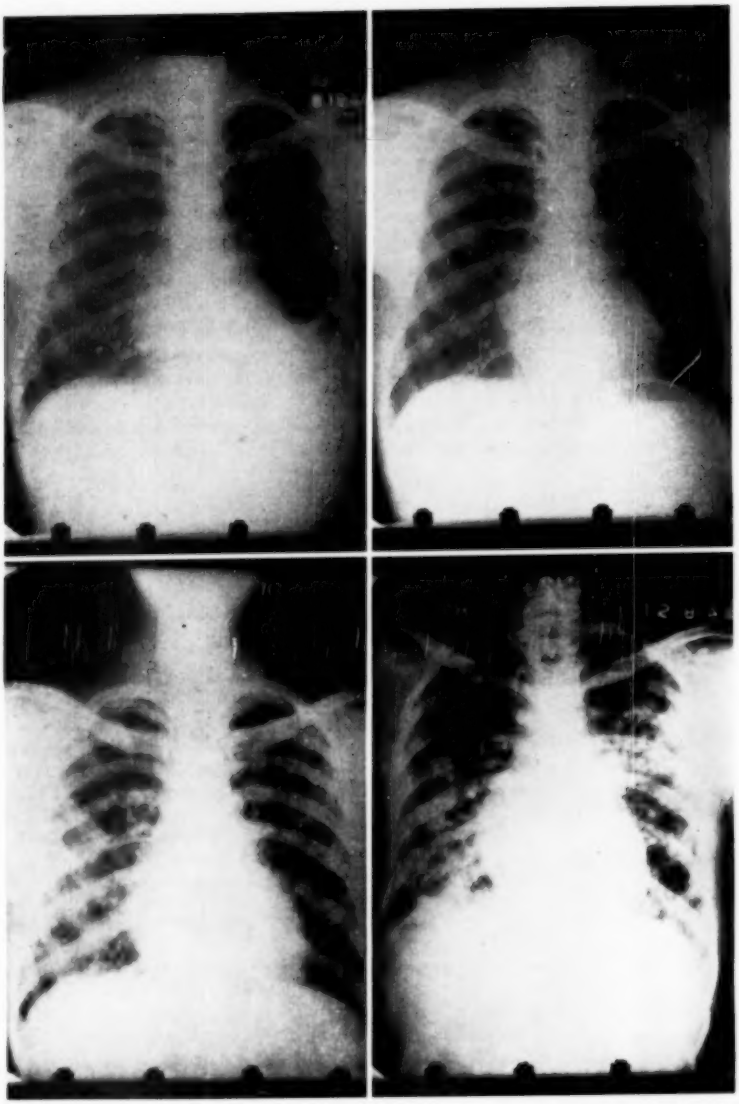


FIG. 1. (Upper left) A pleural effusion in the left hemithorax is noted. Linear infiltrations are present in the second and third intercostal spaces, anteriorly, on the right. The transverse cardiac diameter is enlarged.

FIG. 2. (Upper right) A soft irregular mottling in both lung fields is seen. The pleural effusion noted in the left hemithorax in figure 1 is not present.

FIG. 3. (Lower left) shows an increase of the previously described pulmonary shadows. There is further enlargement of the transverse cardiac diameter.

FIG. 4. (Lower right) Further progression of the pulmonary lesions is noted. There is marked increase of disease in the mid portion of the left pulmonic field. Mottling at the left base is denser, and blunting of the costophrenic sulcus is present.

On admission to this hospital on November 2, 1948, the patient appeared acutely ill, was apprehensive, complained of severe headache and nausea, and coughed up a considerable amount of bloody sputum. A weight loss of 33 pounds was recorded. The oral temperature was 99.4 F. All other findings conformed to those previously recorded. Laboratory tests on blood, urine, and cerebrospinal fluid were all within normal limits. A single positive smear for acid-fast bacilli was obtained in the course of many sputum examinations.

Chest roentgenograms showed increased disease, and on November 8 a course of streptomycin therapy, one gram daily, was begun. Failure to obtain a favorable response to this antibiotic, together with a gradual deterioration of the patient's condition, led to the consideration of carcinomatosis and lymphoblastoma. A Mantoux test was positive with 0.00002 mg. PPD. Roentgenographic studies of the skeletal system revealed no abnormalities, but a chest roentgenogram of December 8 (figure 4) showed progression of the pulmonary lesions. Neurologic studies revealed a positive Babinsky on the left, with a deviation of the tongue to this side. On December 16, dysphagia and hoarseness developed and a paralysis of the left vocal cord was found. Multiple nodules in the scalp were noted. These were firm, discrete, of a cherry-red color, varied from 3 to 10 mm. in diameter, and were freely movable with the skin. At this time, a diagnosis of Kaposi sarcoma was entertained.

Numerous cultures and three guinea pig inoculations were all negative for tubercle bacilli. Several cultures for fungi were negative. Biopsy of an axillary lymph node showed only an hyperplasia of lymphoid tissue.

On January 5, 1949, the temperature rose to 101° F. (rectal), and a septic leukocytosis of 15,000 developed. On January 14 the patient expired.

NECROPSY FINDINGS

Necropsy revealed a tumor with generalized metastases which presented blood-red lesions as the outstanding feature. Tumor growths were found in the following locations: deep tissues of the left neck, right heart, pericardium, lungs, peribronchial and mediastinal lymph nodes, mediastinum, spleen, liver, pancreas, throughout the gastrointestinal tract, kidneys, adrenals, testes, vertebral and femur marrow, and skin of scalp.

Apparently the tumor mass in the left neck was the primary growth, and its histologic feature was duplicated in the many metastases. There were large sinuses filled with blood as well as finer blood capillary mesh work. Muscle, nerves, and other tissues were invaded. Tumor growth within normal veins was found. Such an invasion can account for the extensive metastatic involvement of the right heart and organs of the lesser circulation. In places the tumor cells assumed a long spindle shape, and in other areas that were more cellular the cells tended to be ovoid to polygonal. In these latter areas the vascular nature of the tumor was less apparent, mitotic figures were abundant, and tumor giant cells were present in small numbers.

In the majority of lesions in other tissues, the hemangiomatous nature of the tumor was evident. Some of the liver lesions were not unlike a cavernous hemangioma. The lesions in the scalp closely resembled a typical Kaposi skin tumor.

The most striking gross and microscopic metastatic tumor manifestation was present in the right heart. In two areas of the auricular wall the tumor had penetrated and produced a growth that resembled a vegetation on the inner auricular wall. Tumor nodules behind two leaflets of the pulmonic valve caused an inward bulging of these leaflets and an incompetent valve. A tumor mass that in places measured 3 cm. on cross-section involved the whole of the right ventricle wall, and protruding from the inner wall was an intertwined, stringy, friable mass of tumor tissue which was intermingled with the blood within the ventricle. The microscopic appearance of the tumor strands which were intermingled with the blood varied from solid cords of ovoid or polygonal cells to structures that resembled poorly formed blood vessels filled with blood.

The only evidence at necropsy of tuberculosis was a small, healed, primary complex in the upper lobe of the left lung.

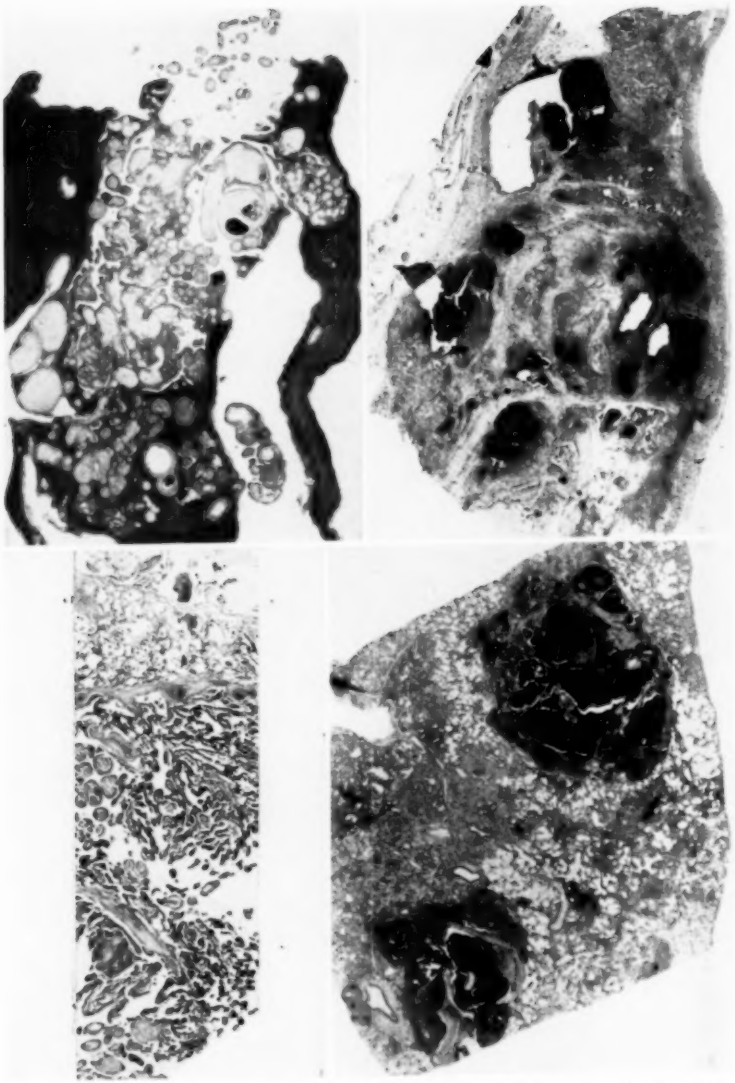


FIG. 5. (Upper left) Fragment of tumor that was intermixed with the blood within the right ventricle. Dark portions are clotted blood. There are solid cords of tumor cells, structures that suggest vessels with organized thrombi, and others that are poorly formed vessels filled with blood. ×10.

FIG. 6. (Upper right) Section from original tumor involving deep tissues of left neck. Dark areas are blood within large spaces. Some blood has dropped out, and such areas suggest the relative size of such sinuses. Tumor growth within normal veins was found in other sections. Tumor had invaded striated muscle, fat tissue, and nerves as well. ×5.

FIG. 7. (Lower left) Section through right ventricular wall. Tumor had grown between and had separated the normal musculature. In this area the ventricular wall was 3 cm. thick. ×5.

FIG. 8. (Lower right) Section from lung showing two tumor metastases. Dark areas are blood within large sinuses. The rest of the lung presents a picture of areas of inhaled blood, old and recent, and of emphysema. Grossly and microscopically these areas resemble chronic passive congestion frequently observed in heart failure. ×5.

The cardiac murmur could be accounted for by the manner in which the tumor growth involved the right ventricle; the pericarditis, by tumor growths within that structure; the dysphagia and paralysis of the vocal cord, by pressure upon an involvement of mediastinal nerves; and the headaches, by direct involvement of nerves in the original tumor mass in the left neck.

The number of metastatic nodules were much fewer in organs of the greater circulation, and this situation may be explained on the basis that blood-borne metastases from the pulmonary veins were a late manifestation of the disease.

DISCUSSION

The pertinent features in this patient were the diagnostic problems presented, the unusual primary site of the tumor, and the rapid course of the disease. The occurrence of cough, bloody sputum, pleural effusion, pericarditis, swollen nodes in the left neck, appearance of progressive bilateral pulmonary shadows, and acid-fast bacilli in smears from sputum naturally led to a diagnosis of tuberculosis early in the course of the disease. Against this diagnosis was the critical condition of the patient without fever or increased sedimentation rate. Failure to respond to streptomycin therapy and to prove the presence of tubercle bacilli by culture or animal inoculation, plus the development of cardiac murmurs and of nerve paralysis, indicated that tuberculosis was not the correct diagnosis. As the condition of the patient rapidly deteriorated, a malignant tumor was suspected and, with the appearance of hemorrhagic tumor nodules in the scalp, Kaposi's sarcoma was suspected. Necropsy revealed the condition to be a generalized hemagio-sarcomatosis originating in the deep tissue of the left neck.

The gross and microscopic features correspond to a Kaposi sarcoma, although the site of the original tumor is unusual. In 1872, Kaposi described a tumor, usually seen in the skin of the lower extremities, and called the condition "idiopathic multiple pigmented sarcoma." Later this tumor became known as a Kaposi sarcoma or multiple idiopathic hemorrhagic sarcoma. There is as yet no unanimity of opinion relative to the exact nature of this disease, as is evident from some thirty-one synonyms applied to it (1). A survey of the literature shows that this type of tumor is most often observed in the skin, but cases have been observed in which the primary tumor occurred in some other organ (2, 3, 4, 5, 6, 7, 8). The patient reported in this paper belongs to the latter category. There can be little question that the type of cell in this case arose from cells whose function is vessel formation.

In general, the disease pursues a chronic course, and generalized metastases, if they occur, are usually a late manifestation. The rapid course in the patient recorded in this paper is unusual.

SUMMARY

A case of generalized hemangio-sarcomatosis with the primary tumor located in the deep tissue of the neck is reported. Grossly and histologically, the tumor resembled a Kaposi sarcoma.

Several clinical manifestations, together with the discovery of acid-fast bacilli in smears of sputum, led to a diagnosis of tuberculosis. Failure to demonstrate the presence of tubercle bacilli by culture or animal inoculation, together with failure to respond to streptomycin and the rapid deterioration of the condition of the patient, led to the consideration of a malignant tumor. The appearance of hemorrhagic tumor nodules in the skin of the scalp late in the illness suggested the diagnosis of Kaposi sarcoma which was confirmed at necropsy.

Death within six months after the first clinical symptoms shows that at times this type of malignancy can be fulminant.

SUMARIO

Caso de Hemangio-Sarcomatosis Generalizada Considerada Erróneamente como Granulia

El caso comunicado es de hemangio-sarcomatosis generalizada, con el tumor primario radicado en el tejido profundo del cuello. Macroscópica e histológicamente, el tumor se parecía al sarcoma de Kaposi.

Varias manifestaciones clínicas, junto con la presencia de bacilos ácidorresistentes en los frotes del esputo, condujeron al diagnóstico de tuberculosis. La falta de descubrimiento de bacilos tuberculosos en cultivos o por inoculación en animales, junto con la falta de respuesta a la estreptomicina y la rápida agravación del estado del enfermo, hicieron pensar en tumor maligno. La aparición de nódulos neoplásicos hemorrágicos en el cuero cabelludo ya avanzada la enfermedad sugirió el diagnóstico de sarcoma de Kaposi, que fué confirmado en la autopsia.

La muerte en término de seis meses de los primeros síntomas clínicos demuestra que esta clase de malignidad puede ser a veces fulminante.

Acknowledgment

The writers wish to express their appreciation to Dr. Edgar Medlar and to Dr. Nicholas D'Esopo for their kindly suggestions in the preparation of this paper.

REFERENCES

(1) MacKee, G. M., and Cipollaro, A. C.: Idiopathic multiple hemorrhagic sarcoma, Am. J. Cancer, 1936, *26*, 1.

(2) Dörffel, J.: Histogenesis of multiple idiopathic hemorrhagic sarcoma of Kaposi, Arch. Dermat. & Syph., 1932, *26*, 608.

(3) DeSalles, J. F.: Hemangioreticuloendothelioma of pericardium, Mem. Inst. Oswaldo Cruz, 1944, *40*, 91.

(4) Aegerter, E. E., and Peale, A. R.: Kaposi's sarcoma: Critical survey, Arch. Path., 1942, *34*, 413.

(5) Stats, D. J.: Visceral manifestations of Kaposi's sarcoma, J. Mt. Sinai Hosp., 1946, *12*, 971.

(6) Tedeschi, C. G., Folsom, H. F., and Carnicelli, T. J.: Visceral Kaposi's disease, Arch. Path., 1947, *43*, 335.

(7) Paolini, R.: Sul sarcoma molteplice primitivo di Kaposi con speciale riguardo alle localizzazione viscerali, Rassegna internaz. clin. e terap., 1927, *8*, 514.

(8) Amsterdam, H. J., Grayzel, D. M., and Louria, A. L.: Hemangio-endothelioblastoma of the heart, Am. Heart J., 1949, *37*, 291.

Editorial

Specific Therapy for Tuberculous Meningitis

It has been four years since Hinshaw and Feldman described the beneficial effect of streptomycin in the treatment of some forms of human tuberculosis. In one of the first of their historical papers the authors describe apparent "cures" of four individuals with tuberculous meningitis[1]. Since that time there have been many reports indicating that streptomycin is, indeed, capable of aiding the infected host to obtain a clinical remission from this otherwise fatal central nervous-system disease. This therapeutic advance, greater actually than could have been anticipated, is not, however, as ideal as superficially appears.

Streptomycin is the most effective tuberculostatic agent available, and it must remain the basic ingredient of any therapeutic regimen designed to treat tuberculous meningitis. Toxicity to it can be entirely ignored in the treatment of meningeal tuberculosis for no single toxic event or combination of them is as serious as the inevitable outcome of the disease without antimicrobial therapy. There is no contraindication to long-term administration of streptomycin, courses of six months being feasible, even practicable. Other tuberculostatic materials, including any one of a number of sulfones, and para-aminosalicylic acid have been added as adjuvant agents to streptomycin. It is not proved that a single one or combination of these has enhanced the total tuberculostatic effect of streptomycin alone, but it is most assuredly true that without streptomycin the use of any or all cannot be expected to alter significantly the outcome of tuberculous meningitis. It remains then for streptomycin to be employed wisely.

Despite the fact that streptomycin is the common denominator in all successfully managed cases, there are certain discrepancies in the reported results which require comment. Among the unexplained facts are the differences in the case fatality rates observed in the various reported series of children with tuberculous meningitis. Doctor Lincoln at New York University-Bellevue has had less than 30 per cent mortality in children[2], and Doctor Cocchi has reported only a 15 per cent mortality in his later series[3]. Both investigators, with their associates, have combined Promizole, or one of the other sulfones, with streptomycin. Less good results have been obtained by one group in Paris[4] and two groups[5, 6] in England with the use of streptomycin alone, the case mortality

[1] Hinshaw *et al.*, J. A. M. A., 1946, *132*, 778.
[2] Lincoln *et al.*, Lancet, 1949, *1*, 707.
[3] Cocchi, C., Riv. clin. pediat., 1948, *46*, 209.
[4] DeBré *et al.*, Brit. J. Med., 1947, *2*, 897.
[5] Smith *et al.*, Lancet, 1948, *1*, 627.
[6] British Medical Research Council, Lancet, 1948, *1*, 582.

rates being 66 per cent, 62 per cent, and approximately 50 per cent, respectively. These marked differences in fatality rates may possibly be due to the addition of a sulfone by Doctors Lincoln and Cocchi. On the other hand, efficacy of treatment in adults has not been enhanced by the addition of either Promin or para-aminosalicylic acid. Similarly, initial, though small, cooperative and controlled studies under the auspices of the Public Health Service have failed to demonstrate significant improvement in the outcome of children treated with streptomycin and a sulfone over those treated with streptomycin alone. The discrepancy (15 to 30 per cent versus 50 to 66 per cent) remains unexplained and further observations must be recorded before the role of sulfones can be assayed accurately.

It is possible that significant difference exists between the disease in children and in adults, a difference not appreciated prior to the use of streptomycin. That this is so is evident when results of antibacterial therapy in each group are studied. Figures for mortality in children range from 15 to 66 per cent. The variation in adults ranges from 50 to 90 per cent. (Only patients followed for at least five months post therapy are included in these calculations.) These are statistically sound figures which indicate that survival occurs more often in children than in adults. The difference may be due to the method of administering streptomycin, to the particular therapeutic regimens utilized, or to the adjuvant use of sulfones. Most pediatricians have given relatively large doses of streptomycin, often in excess of 100 milligrams per kilogram body weight daily intramuscularly, and 100 milligrams intrathecally. This fact may be of some consequence. Save for the first few cases in the Veterans Administration series[7a], the usual daily intramuscular dose for adults has been from 2 to 3 grams (25 to 50 milligrams per kilogram body weight). Prior to disclaiming the efficacy of large daily intramuscular and intrathecal doses, careful observations in a series of adults receiving from 6 to 8 grams daily appear to be in order.

Irrespective of advantages possibly gained by excessive dosage of streptomycin, there still appears to be a difference in the disease according to age grouping. Three possible explanations of this unexpected phenomenon are offered, all speculative yet important perhaps, in further understanding of the problem for the therapeutist.

Tuberculous meningitis in children is, in the majority of instances, closely related to the onset of primary tuberculosis, pulmonary or elsewhere. In the adult, meningitis usually develops in association with acute generalized hematogenous tuberculosis. It is conceivable that the central nervous-system metastases from an active, or even subsiding, primary focus are less numerous than occur as a consequence of the acute, generalized hematogenous type of tuberculosis. Presumably an antibacterial agent can more effectively control a less than overwhelming tuberculosepsis and localized collections of few tubercle bacilli than the situation which usually obtains in the adult who develops tuberculous meningitis following dissemination of great numbers of organisms. It is also possible, in the attempt to explain the difference between childhood and adult

[7a] Bunn, P. A., Am. J. M. Sc., 1948, *216*, 286.

meningitis, that immune body response or other host defense mechanisms are more rapidly mobilized and are more effectual in controlling a primary infection, when supported by proper antimicrobial materials, than they are in the individual afflicted with a chronic illness. It is assuredly true that, without extraordinary activity on the part of the host, streptomycin or any other tuberculostatic agent will not alone, or in combination, achieve eradication of a tuberculous infection in the brain or elsewhere. Ordinarily, the extensiveness of the primary focus is also far less of a problem of repair to the host than a chronic process in bone or lung of the adult—lesions associated with a greater amount of tissue destruction and fibrosis. In any event, the adult, because of ineffectual body defenses or a sudden spread of overwhelming numbers of organisms, or possibly because the pathologic process cannot completely reverse, does not respond as well to therapy for tuberculous meningitis as does the child.

There is yet another unexplained fact that deserves comment. The discrepancy in the reported figures concerning the mortality rate of the disease solely in adults also varies considerably. This may be due to variations in treatment techniques. Modifications of treatment regimens are as numerous as the authors reporting them. It is impossible, indeed, to find a single commonly used schedule, albeit principles of therapy are remarkably similar. The British administer 2 grams of streptomycin daily intramuscularly for six months and 100 milligrams intrathecally daily for six to twelve consecutive weeks[8]. The Belgians report the use of 1.2 to 2 grams per day intramuscularly and from 50 to 100 milligrams intrathecally daily for forty-five days; thereafter intrathecal treatment is given less often but is continued for six months[9]. The Veterans Administration has advocated 2 grams daily intramuscularly and 50 milligrams three times weekly for three to four months[7a]. McDermott and his associates have given 3 grams daily for forty-two to one hundred and twenty days and from 100 to 375 milligrams intrathecally at intervals ranging from twenty-four to seventy-two hours for sixty to ninety days[10]. When compared with the amounts used in children as reported by Lincoln, DeBré, and others, these dosages are small. They are, however, theoretically adequate.

There is unanimity of opinion that the presence of acute generalized hematogenous (miliary) tuberculosis with meningitis reduces significantly the chance for survival. In the Veterans Administration series, for example, less than 10 per cent of cases with combined disease survive, whereas 20 per cent of individuals with meningeal disease alone are saved[7b]. There is no apparent explanation for this observation except that the patient, young or old, must fight two otherwise fatal complications. The very fact that both complications develop, irrespective of focus from which the dissemination occurs, indicates that immunity has been overwhelmed and, presumably, that multiple foci of disease produce almost insurmountable handicaps for the tuberculostatic ability of streptomycin.

[8] Cairns *et al.*, Proc. Roy. Soc. Med., 1949, *42*, 155.
[9] Goidsenhoven *et al.*, Bruxelles-méd., 1949, *54*, 1.
[10] McDermott *et al.*, Ann. Int. Med., 1947, *27*, 769.
[7b] Bunn, P. A., Am. J. M. Sc., 1949 (in press).

It must be emphasized that from 50 to 67 per cent of all adults with miliary tuberculosis will sooner or later develop meningitis. It is apparent that, for preventive reasons, patients under therapy for miliary disease should be subjected to routine periodic cerebrospinal fluid examinations so that the advent of meningitis can be promptly diagnosed and proper changes in the therapeutic program instituted.

In order to draw together other individual advantages of each regimen, a description of what appears to be an ideal treatment outline is offered below. Obviously precision in definitions is impossible. Before any regimen can be evaluated, however, the follow-up period of observation is of signal importance and may partially explain discrepancies in the reported statistics. All patients in the Veterans Administration series, for example, have been observed for a minimum of two years after *completion* of therapy and for almost thirty months following the original diagnosis[7b]. The 10 per cent survival figure in that series can be doubled if the period of observation is shortened by one-half.

It is advisable to administer at least 2 to 3 grams of streptomycin or dihydrostreptomycin intramuscularly in two to four divided doses each day for adults, and 100 milligrams per kilogram per day for children who weigh less than 20 kilograms. It is not possible to state that 6 to 8 grams daily may be indicated. In the writer's opinion, doses larger than 2 to 3 grams daily probably do not offer increased antibacterial protection to the patient. Nevertheless, as present information indicates that survival rates are best in those series in which the largest amounts of streptomycin are administered, it is apparent that further studies in the problem of daily dosage must be made.

In the opinion of most authorities, it is essential to augment intramuscular therapy with direct additions intermittently of extra amounts of streptomycin intrathecally. Fifty to 100 milligrams to adults and 2 milligrams per kilogram per injection to children weighing less than 25 kilograms are needed. When these amounts are injected slowly, serious untoward irritative reactions are reduced to a reasonable minimum.

No precise definition of how long streptomycin should be administered can be proffered. At least one hundred and twenty days' total treatment by parenteral administration seems necessary; less is probably insufficient, and more is not contraindicated. Certainly, treatment periods of six months are worth the expense and trouble if prolonged clinical remissions are not obtained sooner. From the reported experience, at least 40 separate intrathecal injections are necessary and it is recommended that there be daily injections for at least six weeks. Thereafter this minimal therapy may have to be extended if clinical signs and cerebrospinal fluid components have not properly subsided. Certain clinical and laboratory criteria can be utilized to aid partially in the decision to continue or to discontinue intrathecal therapy. Determinations of the sugar and chloride content of the cerebrospinal fluid should be normal, the cell count should be at a steady low level, i.e., below 80 cells per cu.mm. and the value for total protein should be stabilized below 75 milligrams per 100 cc. For as long as streptomycin is given intrathecally, values of the latter two elements may not return to normal

because of the initiative action of streptomycin. All clinical evidence of central nervous-system infection should have disappeared before cessation of therapy is advisable. Following cessation, re-treatment is indicated if the cerebrospinal fluid cell count does not return rather promptly to normal, if the protein does not fall to normal over a period of a few weeks, and whenever clinical infectious relapse becomes obvious.

In the presence of cerebrospinal block, interrupting the spinal flow of cerebrospinal fluid, intracisternal injections of streptomycin are feasible and may be necessary. The same schedule of injections obtains as that suggested above. If a block to the system occurs at a higher level, a surgical procedure (for example, removing ventricular fluid at intervals through surgically established frontal burr holes or short-circuiting the ventricular system[11]) may be warranted.

These rather arbitrary definitions of treatment with streptomycin are not ideal but it appears that they represent basic requirements. Although the addition of adjuvant drugs (para-aminosalicylic acid, Promin, Promizole) may not improve the results, it seems logical to add one or more to the regimen until the question is settled. At least 10 grams of para-aminosalicylic acid daily, given orally for as long as six months, are probably needed, and blood levels of Promin should be maintained at a level of 2 milligrams per 100 cc. Promin can be given orally or parenterally by vein. Untoward side reactions are observed more frequently by the former route. Apparently the material is broken down in the small intestine and the more toxic parent substance, 4-4-diaminodiphenyl sulfone, formed before absorption. It is because of this that Promin by vein is more desirable. Promizole by mouth has some tuberculostatic activity and is considerably less toxic than Promin though given in larger amounts. Although a derivative of 4-4-diaminodiphenyl sulfone, it becomes conjugated in the gastrointestinal tract and is absorbed as a soluble material having antibacterial activity without the associated and expected toxicity of the parent substance.

As the present mortality rate remains high and as all too often a "successful" end result may be a person with serious and irreversible brain damage, some rearrangement of, or addition to, the schedules in the hope of possibly altering the outcome seems appropriate. Study of the pathology of the disease gives two enlightening facts. Presumably streptomycin keeps the infecting organism in a state of bacteriostasis. The majority of deaths are not, therefore, the result of overwhelming proliferation of the bacteria or its toxins. Deaths occur for the most part because of the development of a degenerative hydrocephalus and/or widespread cerebral arteritis and thrombosis which is responsible for severe encephalitis. The British have recently reported the use of streptokinase, added to intrathecally administered streptomycin[12]. As this material has the ability to dissolve fibrin, which when deposited about the brain stem generally results in obstructive hydrocephalus, combined therapy with streptomycin and streptokinase may very well solve, partially at least, the problems of hydrocephalus. Recently the Army has added heparin to the regimen in use, in the hope that

[11] Cairns, H., Brit. J. Med., 1949, *1*, 969.
[12] Cathie, I. A. B., Lancet, 1949, *1*, 441.

continued cerebral arteritis may be held to a minimum, thus preventing brain damage. Both measures deserve immediate and careful study.

One feature of the antagonism between the tubercle bacillus and streptomycin deserves comment. Temporary but impressive clinical remission of tuberculous meningitis is seen in the vast majority of treated cases irrespective of the gravity of the clinical picture with initial treatment; few eventually survive. Relapses are common, almost expected by those treating the disease in any numbers. The failure to observe prolonged or even permanent remission of the disease has often been explained by the emergence to predominance of tubercle bacilli wholly resistant to the antibacterial effect of streptomycin. Although, on the surface, clinical relapse seems closely associated with a change in bacillary susceptibility—it occurs at a period when resistant organisms are expected in other forms of tuberculosis treated with streptomycin—actually it is quite unusual to find streptomycin-resistant tubercle bacilli in the central nervous system even after prolonged exposure to streptomycin. Study of, and explanation for, this basic discrepancy rests with bacteriologists. The clinician, on the other hand, must at all times be consciously aware of three possible ways to circumvent the almost inevitable relapse: (*1*) early and adequate treatment in the hope of eradicating the infection prior to permanent and irreversible brain damage; (*2*) addition of para-aminosalicylic acid, and/or possibly a sulfone, to delay resistance and to offer increased antibacterial protection; and (*3*) prolonging or reinstituting treatment promptly in the face of obvious clinical relapse in the hope that some microorganisms at least remain susceptible and continued treatment useful.

In any event, individuals with tuberculous meningitis deserve specific antimicrobial therapy. It is axiomatic that institution of antibacterial therapy should be as early as possible in the obvious attempt to prevent irreversible brain damage. Observations about promptness of remission of infection following institution of treatment indicate that an impressive response may be delayed for several weeks. Cessation of streptomycin should not then be considered for as long as the patient lives and for as long as clinical central nervous-system infection exists. Though discouraging results have to date been obtained in the treatment of tuberculous meningitis, inroads into the total mortality have been accomplished. Greater strides can be expected with continued strenuous efforts in its therapy.

PAUL A. BUNN

Letters to the Editors

SENSITIVITY TO HISTOPLASMIN

To the Editors of the American Review of Tuberculosis:

The possibility that sensitivity to histoplasmin may be widely distributed in people in other parts of the world than the central Mississippi basin has been suggested recently (Public Health Reports, October 7, 1949). Evidence that this is true was obtained a few years ago from a study of skin sensitivity among patients at the Wm. H. Maybury Sanatorium.

At the suggestion of Dr. Michael L. Furcolow and with the help of Miss Jane Crites, 471 sanatorium patients were tested with 0.1 ml. of a 1:1,000 dilution of histoplasmin (Lot H-15 prepared by Dr. Arden Howell of the Public Health Service). The tests, read at the end of 48 hours, revealed 54 patients with a positive reaction to histoplasmin (area of induration or edema 5 mm. or more in diameter).

Interesting from the epidemiological point of view was the finding of a higher proportion of reactors among patients who had been born in Central Europe. Among 40 patients in this category born in Poland, Rumania, or Jugoslavia, 10 positive reactors to histoplasmin were found. The relative proportion of positive reactors is tabulated below.

Postive Reactors to Histoplasmin
(Maybury patients—1946)

	NUMBER TESTED	NUMBER POSITIVE	PER CENT POSITIVE
Patients born in Central Europe	40	10	25
Patients not born in Central Europe	431	44	10.2
Total	471	54	11.5

The fact that patients of Central European origin showed more than twice the expected proportion of positive reactions to histoplasmin seems significant. The Detroit-born offspring of these patients from Central Europe showed only the average incidence of positive reactors to histoplasmin.

RUBY G. KELLY
C. EUGENE WOODRUFF

WM. H. MAYBURY SANATORIUM
(DETROIT MUNICIPAL TUBERCULOSIS SANATORIUM)
NORTHVILLE, MICHIGAN

December 9, 1949

ENZYMATIC CHARACTERISTICS OF SUSPENSIONS OF DIFFERENT MYCOBACTERIA

To the Editors of the American Review of Tuberculosis:

It was found recently that by mild methods of extraction a material of lipid character can be obtained from virulent but not from avirulent strains of tubercle bacilli (Bloch, H.: J. Exper. Med. [in press]). The removal of this material from the bacterial cells does not interfere with their ability to multiply on culture media. However, virulent bacilli which are phagocytized *in vitro* after the lipid material has been removed no longer inhibit the migration of leukocytes. Inhibition of leukocyte migration *in vitro* was previously found to be characteristic for virulent strains of tubercle bacilli (Allgöwer and Bloch: Am. Rev. Tuberc., 1949, *59*, 562.) Moreover, extracted (but living) bacilli have a reduced state of pathogenicity for animals normally susceptible to tuberculous infections (Bloch, H.: J. Exper. Med. [in press]). On the other hand, when avirulent bacilli were coated with the extracted material, they acquired the ability of inhibiting the migration of leukocytes *in vitro*. From these and other observations, it was concluded that the lipid material is characteristic for virulent strains of tubercle bacilli, that it plays an important part in the relationship between tubercle bacilli and phagocytes, and that it is most likely located at the surface of the bacterial cell surrounding it with a thin cover not present in avirulent strains of tubercle bacilli. This lipid seemed to be responsible for the cord formation (Middlebrook, Dubos, and Pierce: J. Exper. Med., 1947, *86*, 175) of virulent strains and was therefore called "cord factor." While it appeared highly probable that the cord factor coats the surface of the bacterial cell, this location could not be convincingly demonstrated.

In the course of recent experiments concerned with enzymatic processes involved in the metabolism of mycobacteria, an observation was made which sheds more light on the structure of the surface of virulent and avirulent tubercle bacilli and is in support of the view that the cord factor, present only in virulent strains, covers the entire surface of the cell. Furthermore, the observed phenomenon may be useful in differentiating virulent from avirulent strains.

While studying the behavior of different dehydrogenases from tubercle bacilli, using the Thunberg-methylene blue method, it was observed that methylene blue (MB) was much more quickly decolorized by suspensions of avirulent than of virulent organisms. In the presence of different substrates such as dextrose or glycerin, MB was decolorized only by washed suspensions of saprophytic mycobacteria, of avirulent tubercle bacilli, or of BCG strains, whereas comparable amounts of young virulent organisms did not decolorize MB. Even in the absence of added substrates, washed cells of avirulent strains often decolorized MB.

In some instances, the age of the culture was important for the outcome of the experiment. Whereas cultures of avirulent or saprophytic strains decolorized MB at the same rate regardless of the age of the culture, suspensions of BCG and of some strains of virulent bacilli did not decolorize MB as long as they were young or just fully grown, but began to do so when the cultures grew older. This change, however, occurred only when the bacilli were held at 37°C., whereas the decolorizing power of a fully grown culture transferred to the icebox did not change during a long period of time. The time required for an aging culture incubated at 37°C. to become MB—decolorized seemed to some extent to be inversely proportional to the virulence of the strain.

The MB decoloration has the character of an enzymatic action. The temperature

optimum of the reaction is 39°C., its pH optimum ranges between pH 7.0 and 8.0. At 24°C. and 45°C. no reaction takes place. The enzymatic factor responsible for decoloration is inactivated by heating suspensions of washed bacilli to 56°C. for 3 minutes.

Although suspensions of virulent bacilli grown in the usual media do not decolorize MB, the organisms do contain the enzyme. This can be demonstrated by grinding the bacilli with glass powder in a mortar or by extracting them with petroleum ether. Since this last treatment removes the cord factor from their surface (Bloch, H.: J. Exper. Med. (in press)), it may be assumed that the described phenomenon is due to differences in the permeability of the cellular surface of virulent and avirulent strains of tubercle bacilli. The lipid coating of virulent strains does not allow the bacterial enzyme to reach the substrate, or vice versa. This view is further supported by the fact that suspensions of virulent bacilli grown in a medium containing a high concentration of Tween 80 (0.5 per cent) slowly decolorize MB, whereas organisms of the same strain grown in the usual concentration of 0.05 per cent Tween 80 do not.

The test is performed by centrifuging 10 ml. of a fully grown Tween-albumin culture (Dubos and Middlebrook: Am. Rev. Tuberc., 1947, *56*, 334) of tubercle bacilli, washing the organisms with distilled water, resuspending them in 0.5 ml. of 0.06 M phosphate buffer (pH 7.0) containing the substrate, e.g., one per cent dextrose, and transferring them into a Thunberg tube. Five-tenths ml. of MB 1:7,000 is added, the tube immediately cooled to 0°C. and evacuated for two minutes. The evacuated tube is transferred to 38°C. and the decoloration time is measured. Saprophytic mycobacteria decolorize MB completely within one to three minutes, strains of avirulent tubercle bacilli or BCG within two to fifteen minutes, whereas comparable amounts of young virulent strains do not decolorize MB within sixty minutes.

A more detailed report of this phenomenon and its significance will be published later.

HUBERT BLOCH

THE PUBLIC HEALTH RESEARCH INSTITUTE
OF THE CITY OF NEW YORK, INC.
NEW YORK, N. Y.

BOOKS

ERIC WITTKOWER: *A Psychiatrist Looks at Tuberculosis. Pp. 151, 1949, The National Association for the Prevention of Tuberculosis, Tavistock House North, London, Price $3.00*

A practical guide for the pyschological management of tuberculous patients is long overdue. Doctor Wittkower's readable and compact book based on lengthy interviews with 785 tuberculous patients should meet this need. Using clinical illustrations and avoiding psychological jargon, the author describes the reactions of tuberculous patients to their illness, analyzes the factors determining their behavior, and discusses the relevance of emotional factors in the etiology and course of pulmonary tuberculosis.

The observations on the role of sanatorium life in fostering a dependent and rebellious attitude among its inmates should be of interest to all physicians, while patients and doctors alike will appreciate the conclusion that "most of the psychological manifestations of tuberculous patients can be adequately explained on a reactive basis without recourse to the specific and mysterious action of a toxin."

Of special interest is the observation that no uniformity of type could be established in the study of the premorbid personality of 300 tuberculous patients. The following grouping is suggested: (*1*) overtly insecure, 39 per cent; (*2*) rebellious, 12 per cent; (*3*) self-drivers and self-drivers in reverse, 29 per cent; (*4*) conflict harassed, 13 per cent; and (*5*) unclassifiables, 7 per cent.

According to Doctor Wittkower, "situations which rouse aggressiveness or endanger the delicately poised security system of the patients often precede the onset of symptoms of tuberculosis." The insecure overdependent leaning type react disproportionately to the threat of losing or the actual loss of a supporting figure while the rebellious individual overreacts to imposed authority and imposed hardships. All groups appear to have an inordinate need for affection and seem unable "to deal adequately with their aggressive impulses, . . . are prone to turn them against themselves." Representatives of the overtly insecure and of the rebellious types act on the principle, "It serves you right that I suffer," and individuals of the conflict-harassed type on the principle, "I don't deserve to have it any better." Self-drivers have to hurt themselves if they want to hurt those who lashed them into action. Self-damaging features previous to the onset of symptoms of tuberculosis are particularly pronounced in self-drivers in reverse.

The author repeatedly points out, and the reviewer thoroughly agrees, that it is unfortunate no control group has been studied in a similar fashion, for the observations made offer some explanation as to why a person falls ill, and why he falls ill when he does, but do not in any way explain why he falls ill with pulmonary tuberculosis. It is also recognized that the psychological mechanisms discussed do not in any way invalidate the relevance of other etiological factors nor take into account the rule of contact and of massive infection. The emotional upsets associated with relapses appeared to be of a less specific nature than those associated with the onset of the disease. In general, the overtly insecure type take better care of themselves and the course of their disease seems more favorable.

One hundred patients were followed up after two years in an attempt to relate their state of mind at the time of examination with their clinical condition at the time of follow-up. Severe concern and display of psychological defenses interpreted as defiance, resentment, apathy, or overcheerfulness appeared to foreshadow an unfavorable course, whereas those who were less concerned had a more favorable outcome. While much remains to be learned both about the infective agent and the reaction of the host in tuber-

culosis, clinicians will find that Doctor Wittkower's observations have immediate application in the care of tuberculous patients.

BEATRICE BISHOP BERLE

ARTHUR MASSEY: *Modern Trends in Public Health. Pp. x, 581, 1949, Paul B. Hoeber, Inc., New York, Price $12.50*

Although this symposium by 23 different authors does not claim to cover the whole field of public health, the reader will find comprehensive statements written by men who are well known and competent to discuss present accomplishments and future needs. One is struck, as usual, by the greater tendency of the British to turn at once to official organization for the solutions to problems in health, but this may be due in part to the compactness of their jurisdiction and their longer experience with official health and welfare agencies.

The book has no organization except that articles are arranged alphabetically by authors' names, but the brief foreword by Wilson Jameson provides some historical framework, and aids in obtaining the proper perspective. The developmental progress of public health is further covered by F. A. E. Crew in the chapter on "Social Medicine as an Academic Discipline." He says, "... public health has latterly become freed from the domination of those laboratory sciences which deal with the phenomena found in man's external physical world—physics, chemistry, meteorology and bacteriology—and is now eagerly seeking to resume its earlier direction of development as a social science."

Nevertheless, man's physical world is well covered in several other chapters, including "Recent Laboratory Contributions to Epidemiology" by Robert Cruickshank, "The Combat with Atmospheric Pollution" by J. Johnstone Jervis, and "Housing and the Home" by J. M. Mackintosh.

With American public health nursing trends in mind, John D. Kershaw's comment is pertinent, "The health visitor of the future seems likely to be a general practitioner social nurse, engaged in child welfare, school health work, the social care of the aged, hospital and after-care social work and miscellaneous social work associated with epidemiology, housing, and every other branch of public welfare."

Space does not permit individual comment on the essays covering such important fields as chronic rheumatism, tuberculosis, dental health, mental health, occupational health, and health education, but the problems and trends as reviewed by our British friends are strikingly similar to those observed in this country.

FLOYD M. FELDMANN

AMERICAN TRUDEAU SOCIETY

Medical Section of the National Tuberculosis Association

Report of the Laboratory Subcommittee of the Committee on Medical Research and Therapy, and of the Subcommittee on Evaluation of Laboratory Procedures of the Committee on Revision of Diagnostic Standards

Laboratory Subcommittee

Dr. Guy P. Youmans, Chairman
Dr. Joseph D. Aronson
Dr. Martin Cummings
Dr. Stuart Mudd
Dr. C. Richard Smith
Dr. Arthur J. Vorwald
Dr. C. Eugene Woodruff
Dr. Alfred G. Karlson

Subcommittee on Evaluation of Laboratory Procedures

Dr. C. Eugene Woodruff, Chairman
Dr. Emil Bogen
Dr. Edwin A. Doane
Dr. Michael M. Furkelow
Dr. Max B. Lurie
Dr. Edgar Medlar
Mr. William Steenken, Jr. (NTA).

Introduction

Tuberculosis as a chronic infectious disease unresponsive to specific biologic therapy has challenged many investigators to search for effective chemotherapeutic agents. Nevertheless, special biological attributes of *Mycobacterium tuberculosis* and special features of the pathogenesis of tuberculosis postponed even the present modest measure of success in chemotherapy of tuberculosis until after the arsenicals, sulfonamides, and penicillin had revolutionized the therapy of other bacterial infections. The discovery of streptomycin and its application in tuberculosis have greatly stimulated the search for new tuberculostatic agents which might conceivably be of even greater potency than streptomycin, or which at least might supplement this antibiotic in some form of combined therapy.

Special features of tuberculosis necessitate somewhat special methods of evaluation of tuberculostatic agents. The present report is primarily intended as an exposition of currently proved methods of evaluation of tuberculostatic agents *in vitro* and in the mouse, guinea pig, and rabbit as experimental animals. Such methods are undergoing constant revision and adaptation. Presentation of explicit procedures in this report is intended as a guide to principle and procedure as presently understood, but is obviously subject to modification and elaboration as experience and understanding grow.

Although the pathogenesis of each infectious disease presents features characteristic of itself, these must be regarded as modifications of more general principles of bacteriology

and immunology and of broad patterns of host-parasite interrelationships which are common to all infections. It is then reasonable to hope that present-day empirical chemotherapy in tuberculosis, as in other infectious diseases, may gradually be replaced by a more rational chemotherapy. Such rational chemotherapy would be based upon a profound understanding of pathogenesis, of the metabolism of mycobacteria from the standpoint of synthesis of amino acids, proteins, coenzymes, nucleic acids, et cetera, energy exchange, and the interconversion of metabolites, alteration in metabolic inhibitors, and the genetics of mycobacteria. Fundamental metabolic studies of *M. tuberculosis* are currently at a much less developed stage than are such studies with various non-acid-fast pathogenic bacteria. A section is therefore included in this report on a method of determining growth rate of *M. tuberculosis* under defined conditions. It is hoped that this procedure may serve to illustrate to bacteriologists and biochemists that these microorganisms are susceptible to quantitative metabolic study, and may point out to specialists in tuberculosis one means of conducting investigations of more fundamental aspects of bacterial physiology as a basis for a rational chemotherapy.

Before beginning experimental testing of any agent proposed for the treatment of tuberculosis, the process of reasoning upon which its alleged effectiveness is based should be subjected to logical analysis. The facts asserted to support the suggested substance should be verified and the validity of conclusions drawn from them carefully examined. Measures based upon fallacious and obsolete notions regarding the bacteriology, pathogenesis, and course of tuberculosis may often be eliminated by such rational considerations. If a medication purporting to kill the tubercle bacillus in the body is found incapable of affecting it even *in vitro*, and if it is harmful to the tissues of the patient in concentrations that are still ineffective against the germs, there seems little basis for further investigation. Thus, since tubercle bacilli may be grown from sputum after treatment with alkalis, acids, oxidizing or reducing substances, which would destroy any human tissues, these substances offer little prospect of providing a bactericidal effect within the body. The use of phenol and other toxic drugs which might perhaps injure the bacillus but which would undoubtedly harm the patient's own tissues may be rejected without prolonged experimentation. Agents of unknown composition should be particularly scrutinized for the possibility of such dangerous effects.

In Vitro Tests

Many investigators have attempted to obtain information which might aid in the identification of potential antituberculous agents by determining the bacteriostatic effect of compounds on the tubercle bacillus *in vitro*. The rationale for this procedure is based on the premise that the action of an antimicrobial agent *in vivo* is due to its direct effect upon the infecting organism. That this is a reasonable assumption is illustrated by the action of the sulfonamides, the sulfones, and several antibiotics on tubercle bacilli and other bacteria. Some investigators have felt that this *in vitro* procedure was of little value, however, because no correlation could be demonstrated between bacteriostatic activity *in vitro* and therapeutic action *in vivo*. Many substances which are highly bacteriostatic *in vitro* are not effective chemotherapeutic agents *in vivo*, and in some cases substances which are inactive bacteriostatically *in vitro* have some chemotherapeutic action *in vivo*. The presence or absence of a correlation between *in vitro* bacteriostatic activity and *in vivo* chemotherapeutic activity, however, depends upon the manner in which the *in vitro* tests are conducted and also upon the interpretation of the results, since many factors may tend either to reduce or increase the effectiveness of a compound *in vivo*. For example, a substance which is highly active *in vitro*, in a protein-free medium, may be inactive *in*

vivo because it combines with protein and therefore is not free to act upon the invading bacteria. Compounds given to experimental animals by the oral route may not be absorbed in sufficient quantities from the intestinal tract to give a therapeutic concentration at the site of the lesion. Even though absorbed, certain compounds may be so rapidly excreted from the body that an effective concentration cannot be maintained. Substances may be inactivated metabolically so rapidly within the body that no therapeutic effect can be obtained. The toxicity of a compound for tissue cells may be so great that therapeutic doses cannot be administered. Certain compounds inactive *in vitro* may be broken down *in vivo* to compounds which would be active *in vitro*, i.e., prontosil and promin.

All of the above factors must be considered when attempts are made to correlate *in vitro* bacteriostatic action with antimicrobial action *in vivo*. The result will be that the vast majority of substances active *in vitro* will be rendered inactive *in vivo* by virtue of one or more of these factors (1, 2).

In vitro bacteriostatic tests using tubercle bacilli are difficult to perform and to standardize because of the peculiar nature of the organism and the slowness of its growth. Such standardization is essential, however, for the proper interpretation of results. This is well illustrated by the contradictory reports in the literature regarding the *in vitro* bacteriostatic effect of similar chemical substances. These divergent results are probably due primarily to the different methods employed by various investigators.

When properly performed and evaluated, *in vitro* bacteriostatic tests will serve three useful purposes: (*1*) the detection of highly bacteriostatic agents; (*2*) the elimination of non-bacteriostatic substances; and (*3*) will show the relation between chemical structure and tuberculostatic activity permitting, possibly, the synthesis of more active or more pharmacologically suitable derivatives.

It follows that, if information is desired from *in vitro* bacteriostatic tests which will permit the correlation of bacteriostatic activity with chemical structure and the elimination from consideration of inactive compounds, it is necessary to conduct the tests under conditions which will detect minimal bacteriostatic activity. For the successful accomplishment of this purpose, certain factors must be carefully considered. These are: (*1*) the nature of the medium; (*2*) the nature of the test organism; (*3*) the type of growth of tubercle bacilli used for the test; (*4*) the size of the inoculum employed; and (*5*) the nature of the substance being tested. A general discussion of these factors will first be given, following which the recommended *in vitro* procedure will be given in detail.

Medium: A wide variety of media have been used for the testing of tuberculostatic agents. These can be divided into two general types: (*1*) complex nutrient media, and (*2*) synthetic nutrient media (3–30). The latter probably should be called chemically defined media, since all of the constituents are known. Both types of media have been employed in both solid and liquid form.

The complex nutrient media which have been employed for bacteriostatic tests have been for the most part those used for the isolation of tubercle bacilli from clinical material. They consist, when used in the solid form, of a nutrient base to which is added egg yolk, glycerol, and often a variety of other ingredients. Agar is sometimes used as a solidifying agent. When used in the liquid form, the egg yolk is omitted. These media have the over-all disadvantage of being of unknown chemical composition, making it impossible for them to be duplicated accurately. Moreover, it is impossible to define accurately the effect of the various ingredients in the medium either on the growth of the tubercle bacillus or upon the substance which is being tested.

It should be apparent that any medium, or substance within a medium, which stimulates markedly the growth of the tubercle bacillus may obscure the effect of slightly bac-

teriostatic substances. The presence of protein is a serious disadvantage because of its ability to combine with and render many compounds non-bacteriostatic. This is particularly well illustrated by the action of the sulfonamides and sulfones in enriched media. In nutrient broth, or a medium containing protein, frequently more than one hundred times the amount of sulfonamide is needed for bacteriostasis as is necessary in a synthetic medium. The sulfones also are affected in this manner. These media also may contain specific substances which antagonize the bacteriostatic activity of certain compounds. When it is important to determine the effect of protein on the bacteriostatic activity of a compound, this can be done more accurately by adding whole serum or protein to a chemically defined medium, rather than by using a nutrient medium. In this manner the role of the serum or protein alone on the bacteriostatic activity can be determined.

Another disadvantage of these complex media is that when used in the solid form it is frequently difficult to get the compound to be tested into solution and evenly distributed throughout the medium. Furthermore, the use of agar is sometimes necessary and agar in itself has a slight bacteriostatic action on tubercle bacilli (15). When inspissated egg yolk media are employed, the inspissating temperature may alter or destroy the compound under study. These media also are more difficult to prepare, with a resulting lack of economy in time and effort.

Chemically defined media, on the other hand, are easily prepared and can be duplicated readily. The rate of growth of tubercle bacilli in these media can be determined and is fairly constant. Since the ingredients are known, those substances can be avoided which in complex nutrient media tend to affect the bacteriostatic activity of the substances being tested. There are a variety of these chemically defined media which have been used for the growth of tubercle bacilli, including Proskauer and Beck, Long's medium, Kirchner's medium, Sauton's medium et cetera. Any one of these can be used for the testing of tuberculostatic agents. These should be used in the liquid form since the addition of agar has the disadvantage mentioned.

A semi-synthetic medium which recently has been used widely is the one proposed by Dubos, which contains a synthetic nonionic detergent Tween 80 and purified bovine serum albumin (31). It must be recognized that Tween 80, however, may frequently either markedly increase or reduce the bacteriostatic activity of certain antibiotics and synthetic organic compounds (32–38).

The nature of the substance being tested, as will be pointed out later, determines to a considerable extent the methods which should be used. The above statements apply primarily to synthetic organic compounds and the testing of antibiotic filtrates or concentrates. When attempting to isolate, or screen, cultures of microorganisms for their ability to produce tuberculostatic antibiotics, complex nutrient agar media may be necessary (39).

Test organism: It would appear axiomatic that, in a search for a chemotherapeutic agent, the test organism should be identical with the bacterium which causes the disease which we wish to treat. However, many investigators have used rapidly growing avirulent mycobacteria for tuberculostatic tests. The reason for the use of such organisms is readily understood. Virulent human-type tubercle bacilli grow very slowly, are difficult to handle and there is, of course, some danger involved in the handling of virulent organisms. These reasons, however, have no validity unless it can be shown that these rapidly growing acid-fast bacilli are equally susceptible to tuberculostatic activity of all known chemotherapeutic agents. This is not the case (20, 40). Especially to be condemned is the use of the A.T.C.C. strain no. 607 for results obtained with this strain frequently differ markedly from those obtained with virulent human-type tubercle bacilli (20, 40).

Amount of inoculum: In bacteriostatic tests using virulent human-type tubercle bacilli, the amount of inoculum employed is very important. The inoculum should not be too large because even the bacteriostatic activity *in vitro* of known effective chemotherapeutic agents may thus be obscured. This is the result of the fact that the bacteriostatic activity of many substances is inversely proportional to the number of bacteria present. In such cases, the greater the number of organisms the less the bacteriostatic action or, put in another manner, the greater the number of organisms the greater the amount of compound that is necessary to obtain bacteriostasis. The reason for this phenomenon is not apparent, but it must be taken into consideration. On the other hand, the amount of inoculum should not be too small, since this extends the incubation period necessary for adequate growth and therefore unduly prolongs the test.

Type of growth: Tubercle bacilli are aerobic organisms and for many years were routinely cultivated on the surface of liquid synthetic medium. While growth previously had been observed in the depths of liquid media, this was not considered a practical method for the propagation of these organisms. More recent work has demonstrated that tubercle bacilli grow just as well, if not better, in the depth of the medium as on the surface. Thus, a choice of two methods is provided for growing tubercle bacilli when performing tuberculostatic tests.

Many investigators have utilized surface growth, but this technique has many serious disadvantages. Since a large surface area is desirable to provide for growth of the tubercle bacilli, flasks containing from 50 to 100 ml. of media in which is incorporated the test substance are inoculated on the surface with a flake of tubercle bacilli. The tuberculostatic activity of the substance under test is measured in terms of inhibition of the growth of this surface inoculum. That this method is laborious, slow, and cumbersome should be apparent since it requires relatively large volumes of medium and unsuitable containers that are difficult to handle. The method is also far from precise since it is impossible to use a measured inoculum for each flask. Moreover, the inoculum is frequently far larger than should be used for these tests. In transferring flakes of pellicle growth to the test medium, different portions of the pellicle growth are used. There is no assurance that all of the organisms present in a surface pellicle growth of tubercle bacilli are physiologically the same. This means that different concentrations of the test compound may quite possibly be inoculated with organisms which vary from each other. This might result in erroneous or nonuniform results. Such tests, because of the slow surface growth, frequently require a more prolonged period of incubation than is desirable.

It is possible by utilizing the subsurface (deep-seated or submerged) growth of tubercle bacilli to avoid all these objectionable features. The medium can be dispensed in tubes in volumes as small as 4 or 5 ml., thus reducing the amount of medium necessary. Each tube can be inoculated with the same amount of a homogeneous suspension of tubercle bacilli. An inoculum of any desired amount can be employed and can be duplicated with reasonable accuracy from one experiment to another. The incubation period under these conditions is also much shorter. The results of the tests can be read in 7 to 14 days. The method is therefore much more rapid and the results are more uniform and reproducible. Tubercle bacilli under these conditions grow on the bottom and often up the sides of the tubes. There is no tendency for them to disperse throughout the medium. Growth can be detected easily by observing the bottom of the tube or by shaking the tube and looking for the typical masses of tubercle bacilli which then swirl through the medium in the form of flakes. The appearance of this growth is so typical that it can, with a little experience, be readily distinguished from most contaminants.

Nature of substance being tested: The nature of the substance being tested also should

be taken into consideration. Organic compounds of known composition can be weighed and utilized in known concentrations and, by employing a chemically defined medium, it is possible to define accurately the effect of the organic compound upon the growth of tubercle bacilli. Care should be taken, however, to take into consideration the solubility and stability of such substances. Many are very insoluble even in low concentration. When in doubt regarding the stability of a compound, it should *not* be added to the medium before sterilization in the autoclave. Compounds of known heat stability can of course be sterilized in the autoclave following dilution in the medium. However, others may have to be passed through suitable bacteria-tight filters and added to the sterilized medium.

Many tuberculostatic agents are derived from plant or animal sources (antibiotics). These are seldom available in a pure form when the initial information on their antibiotic activity is desired, since they usually consist of filtrates, or concentrates of filtrates, obtained from cultures of microorganisms. These substances can be added to a synthetic medium in the same manner as the organic compound, and the result expressed in terms of the least amount which completely inhibits growth. Comparative tests with a pure antibiotic of known potency, such as streptomycin, will help in the evaluation.

It is frequently desirable to test directly cultures of various microorganisms to determine whether they produce any growth-inhibiting substances for the tubercle bacilli. This can be done in the conventional manner utilizing streak plate cultures of these organisms and cross-streaking with virulent human-type tubercle bacilli. The medium to be used in this case should be the one which best promotes the growth of the organisms being tested for its antibiotic production. A wide variety of very complex media can be employed, and the majority of these will support the growth of virulent human-type tubercle bacilli, providing 1.0 or 2.0 per cent concentration of glycerol is added and the hydrogen-ion concentration is approximately neutral (39).

PROCEDURE FOR PERFORMING IN VITRO TUBERCULOSTATIC TESTS

The following detailed procedure for the performance of *in vitro* tuberculostatic tests[8] is given as an example of a method which takes into consideration the previously mentioned factors (2, 19–24, 38).

Medium: The medium is a modified Proskauer and Beck chemically defined medium which has the following composition:

Asparagin	0.5 per cent
Monopotassium phosphate	0.5 per cent
Potassium sulfate	0.05 per cent
Glycerol (reagent grade)	2.0 per cent
Magnesium citrate	0.15 per cent

These ingredients are weighed out on the basis of the anhydrous material and are dissolved in the order given in water redistilled from glass. The use of redistilled water may or may not be essential, depending upon the purity of the distilled water available. Since distilled water frequently contains substances which are somewhat inhibitory to the growth of tubercle bacilli, however, more uniform results probably will be obtained if redistilled water is used. Each ingredient is completely dissolved in the distilled water before the addition of the next. Following the addition of the glycerol, the hydrogen-ion concentration is adjusted accurately to pH 7.0 with 40 per cent sodium hydroxide. Following this, the magnesium citrate is added and completely dissolved. This medium can be sterilized in the autoclave at 15 lbs. pressure for 20 minutes. A precipitate may sometimes

appear following autoclaving. This usually is due either to the inadvertent use of too high a temperature, too long a time of sterilization, or to the employment of certain batches of magnesium citrate. The precipitate is composed of magnesium phosphate and can be removed by filtration through filter paper. The resulting clear medium can be resterilized and employed for the tests without endangering the results. As previously mentioned, other chemically defined media such as Long's, Kirchner's, et cetera, may also be employed.

Glassware: It is important that all glassware used be as clean as possible. This can be accomplished by washing thoroughly with soap and water or with a suitable detergent preparation, rinsing seven or eight times in tap water and three or four times with distilled water. Cleaning solution can be used if desired, if the tubes are adequately rinsed. The size of the test tubes used is not critical providing the volume of the medium is adjusted to the size of the tube. Pyrex tubes measuring 150 by 20 mm. containing no more than 5.0 ml. of medium are recommended. If larger tubes are employed, the amount of medium can be the same or increased proportionally if desired. Smaller tubes can be used if the amount of medium is reduced. The use of aluminum caps instead of cotton plugs is highly desirable, since they are more convenient and serve adequately to prevent evaporation. If cotton plugs are used, the tubes should be covered with waxed paper or parafilm to reduce loss by evaporation. Cotton plugs should not be sealed with paraffin. All glassware, including pipettes, should be sterilized in the autoclave at a pressure of 20 pounds for 20 minutes.

Preparation of drug dilutions: Any initial concentration may be employed that is desired, although a concentration of 10 mg. per 100 cc. is for most purposes probably the highest necessary. Compounds which are soluble in water may be dissolved directly in the medium. The more insoluble compounds may be first dissolved in 1.0 or 2.0 cc. of ethyl alcohol, acetone, ether, propylene glycol, 0.1 N. sodium hydroxide, or 0.1 N. hydrochloric acid, depending on the substance in which they are most soluble. These then are diluted with the medium to make the desired initial concentration. In this manner, some substances can be dissolved which will not go into solution in the medium directly. Compounds which even with this procedure will not go into solution and which are heat-stable may be heated to boiling for 5 minutes, and the subsequent dilutions made while the solution is still warm. Compounds which will not go completely into solution in the desired concentration, regardless of treatment, either should be tested only in those concentrations which will go into solution, or a saturated solution can be prepared and employed for the preparation of the dilutions. Serial dilutions in which each concentration is one-half of the preceding one are sufficiently accurate for most purposes. Heat-stable compounds can be diluted and then sterilized in the autoclave at 15 lbs. pressure for 20 minutes. Heat-labile compounds should be filtered through a suitable bacteriological filter (sintered glass is preferable) and diluted aseptically in the medium.

Test organism: In view of the desirability for standardization, the H37Rv strain of *M. tuberculosis* var. hominis is recommended for all routine tests. This is a strain of standard virulence with a known history, and results are already available using this organism with a wide variety of tuberculostatic agents. Tests employing other strains of virulent human-type tubercle bacilli should be conducted when information is desired regarding the range of activity of a known tuberculostatic agent.

The number of tubercle bacilli to be employed in tuberculostatic tests is important for the reasons previously mentioned. It is recommended that approximately 0.01 mg. wet weight of a fine suspension of the H37Rv strain per ml. of medium be employed.

Suspensions for inoculation can be prepared in two ways: (*1*) A portion of a 2- to 3-week pellicle surface growth obtained from the same synthetic medium can be placed in a

sterile mortar and ground with a sterile pestle. During the grinding process the culture should be diluted gradually either with the synthetic medium or with 0.01 molar phosphate buffer solution until a fairly heavy suspension is obtained. The suspension is then transferred to a sterile test tube and allowed to stand at room temperature for 30 minutes. At the end of this time, the larger clumps will have settled out, leaving a relatively fine homogeneous supernatant suspension, following which it can be transferred by aspiration or decantation to a second sterile tube. In order to obtain uniform results, the same care is necessary in maintaining the stock cultures as is described for the maintenance of virulence in the section on the test organism to be used for the infection of mice (page 285).

(*2*) Cultures of H37Rv strain grown for 7 to 10 days in Dubos medium (31) can be employed after being diluted to the desired concentration. The small amount of Tween 80 and albumin contained in this inoculum will not affect the results seriously.

Before inoculation of the drug dilutions, the suspensions of tubercle bacilli must be standardized. This can be done by photometric turbidity determinations, or by centrifugation in Hopkins vaccine centrifuge tubes. In the latter method, the packed tubercle bacilli in the tip of the tube are measured in terms of cubic millimeters and each cubic millimeter is assigned arbitrarily a wet weight of 1.0 mg. Following standardization, the suspension is diluted with synthetic medium or 0.01 molar phosphate buffer so that when inoculated the drug dilution tubes will contain 0.01 mg. of tubercle bacilli per ml. of medium.

Care must be taken when inoculating to deliver the same number of tubercle bacilli to each tube in the series of drug dilutions. Suspensions of tubercle bacilli tend to settle out even in pipettes. A dropping device in which the desired inoculum is contained in one or two drops of diluent and in which the bacterial suspension is kept agitated during the inoculating process is far more accurate and convenient.

Time and temperature of incubation: Following inoculation, all tubes are incubated at 37° C. Growth usually will begin to appear, using the above medium and inoculum, within 3 or 4 days as a very small flaky deposit on the bottom of the tube. As incubation continues, the amount of growth increases and the results can be read more easily following a period of about 14 days' incubation. At this time the tubes should be examined, and the bacteriostatic activity of each compound recorded in terms of the least amount which completely prevents the growth of the tubercle bacillus. In addition, by comparison with the amount of growth in the control tubes, zones of partial inhibition of growth can be detected.

The error inherent in the method described above is a plus or minus one dilution. However, experience has shown that with careful performance it is usually much less. It is wise, however, to include with each test some compound of which the tuberculostatic activity is known. For synthetic organic compounds, this can be one of the sulfones, preferably 4,4′-diamino-diphenyl-sulfone, or para-aminosalicylic acid. Performance of these compounds of known activity in any given test will serve as an additional check upon the technique. When testing antibiotics, streptomycin makes a very suitable control.

It is desirable also to determine the bacteriostatic activity of substances in the presence of serum or protein, since compounds which are rendered non-bacteriostatic by the presence of serum should not be considered seriously for *in vivo* therapeutic trial. This can be done by incorporating sterile beef, human, horse, or other mammalian serum in the medium in a concentration of 10 per cent. The tests are performed as described above except that all dilutions must, of necessity, be performed aseptically. Crystalline bovine albumin or purified bovine albumin (Fraction V) can be employed in a concentration of 0.5 per cent instead of serum.

If investigators are interested in the compounds only for their possible usefulness in the treatment of tuberculosis, the initial *in vitro* test may be done only in the presence of serum or protein.

In Vivo Tests

In vivo tuberculostatic tests involve the determination of the ability of a given substance, or combination of substances, to retard or suppress the course of the infection in animals artificially infected with virulent tubercle bacilli.

A wide variety of experimental animals have at one time or another been employed for *in vivo* tuberculostatic tests. These include guinea pigs, mice, rabbits, rats, hamsters, and the developing chick embryo.

Guinea pigs and mice, because of their availability and known response to infection with virulent tubercle bacilli, have been used most extensively.

Selection of animals: The animals should be procured, if possible, from one particular source, in order to ensure uniformity in size, vigor, and health. If, however, they are obtained from various dealers, each shipment of animals must be examined for evidence of infectious diseases and for malnutrition. In fact, newly purchased animals should be placed under observation for a week or two, or even longer if possible. This will give sufficient time for the recognition of any unhealthy signs, and the interval will allow for tuberculin testing of rabbits or guinea pigs in order to exclude the presence of spontaneous tuberculosis. Furthermore, it will enable the animals to become accustomed to new surroundings and diet.

Diet and management: Throughout the course of the experiment, the diet should be uniform to prevent loss of weight, which commonly occurs when a change is made in the type of food. Since most experiments run for several weeks or months the cages should be cleaned at least once a week so that a fair degree of dryness may be maintained. Also, the danger and spread of infection is lessened if few animals are kept in a cage. The ideal procedure is to provide a cage for each animal.

Test organism: The infecting agent for mice and guinea pigs should be virulent human tubercle bacilli. Because of its widespread use, its known history of some forty-five years, and standard virulence, the H37Rv strain is recommended. For rabbits, the well-known Ravenel (virulent bovine) strain should be employed. Virulence of the H37Rv and the Ravenel can be most effectively maintained by surface growth on the modified Proskauer and Beck synthetic medium. Subcultures should be made at least every two weeks, and care must be taken to utilize the filmy type of growth for transfer. Frequently, islands of a piled-up more waxy type of growth will appear in the flasks. These organisms may be less virulent and, therefore, should not be used. The suspensions used for inoculating mice, guinea pigs, or rabbits are prepared and standardized in exactly the same manner as that described for the preparation and standardization of inocula for the *in vitro* tests.

In laboratories where facilities are not available for the maintenance of standard virulence of cultures, it is recommended that such cultures be obtained from the Standard Culture Depot of the National Tuberculosis Association at The Trudeau Laboratory, Trudeau, New York.

Any findings with the laboratory strains of tubercle bacilli may profitably be checked with freshly isolated virulent strains.

Administration of drugs: Depending upon their nature, drugs may be administered either orally or subcutaneously. The simplest but not the most exacting method for the administration of drugs orally is the drug-diet method in which the drug is mixed with the diet and the mice or guinea pigs allowed to partake of this ad libitum. Care must be

taken to see that the drug and food are finely ground and thoroughly mixed to avoid the possibility of the mice or the guinea pigs being able to make any selection between the two. A convenient form of powdered food for mice can be prepared by grinding standard mouse-ration pellets, following which the material is passed through a fine screen to eliminate large particles. The drug in a finely powdered form is added and thoroughly mixed with the diet, preferably in a mixing machine. It is desirable to administer the drug diet to the mice or guinea pigs housed in individual cages with the food in containers of such design that scattering or pollution of the food cannot occur. This permits the determination of the amount of food, and consequently the amount of drug, ingested per day. Such data are sometimes very valuable. However, animals of the same sex may be housed in groups, preferably not to exceed a total of 5, and fed the diet in such a manner that all are able to eat as much as they desire. This does not permit the calculation of the drug intake, but therapeutic results can be obtained which compare favorably with those in which the first method is employed.

A more laborious method of administering drugs orally is by the use of a medicine dropper or a syringe which has a large-bore needle with a blunt end. It is necessary to resort to this method when only small amounts of drugs are available, or if an animal becomes sick and refuses to eat, an occurrence which is frequently observed when the disease is far advanced.

It is necessary to administer some substances, such as streptomycin, subcutaneously in solution. The injections can be made most easily under the loose skin of the back. If care is exercised in handling, mice and guinea pigs will readily tolerate 4 or more such injections per day, providing the volume employed is not too large.

Controls: In testing for the possible chemotherapeutic action of any substance in experimental animals, the controls comprise one of the most essential features of the experiment. An equal number of untreated infected mice or guinea pigs should be employed. It is essential that these control animals be of approximately the same weight and age as the treated animals. It is highly desirable in any such experiment that animals for the treated and control groups be selected in a random manner. The controls should be maintained under the same conditions as the treated animals, except that they receive none of the drug being tested. When testing compounds by the drug-diet method, another series of controls should consist of animals which are receiving in their diet a substance of known chemotherapeutic value. Para-aminosalicylic acid is the most suitable for this purpose and should be administered mixed with the diet in a concentration of 1 per cent.

With substances that are given in solution subcutaneously, a similar series of controls which are treated with 0.75 to 3.0 mg. of streptomycin per day should be used. The latter controls serve to eliminate the possibility of not detecting an active substance when conditions beyond the control of the investigator operate to reduce the effectiveness of the substances. They also serve to give an idea of the value of the unknown substance as compared with the activity of a known compound. A series of control infected animals should also be injected with the diluent alone. These will serve to determine the effect of the diluent and the repeated handling on the course of the infection.

Assay or chemical determination of drug: In cases where chemical or assay methods are available for the determination of an antibiotic or a chemotherapeutic agent, blood can be removed by cardiac puncture from the guinea pig or the mouse, and from the lateral ear vein of the rabbit.

When an agent is found to be effective against tuberculosis in experimental animals, as well as against the tubercle bacillus *in vitro*, extensive additional observations are immediately indicated. The initial observations are usually made with the largest feasible

dosage and the optimal conditions for observing any effect. Further experiment should be made to determine the minimal and the optimal, as well as the maximal, individual dose and frequency of administration, and the length of time that treatment should be continued. The effect of various routes or modes of administration and of other factors affecting the agent and its effectiveness on lesions of various types and locations should also be investigated. Average lethal single doses and courses of treatment should be determined on nontuberculous animals. Any other effects, acute, delayed, or chronic, should be studied by clinical observation of treated animals, functional tests, and microscopic examination of organs or tissues to avoid endangering human patients. When an agent has been shown to possess promise as a result of such thorough experimental investigation, and it has been determined that the treatment carries little or no risk as compared with a fair probability of therapeutic effectiveness, human trial is then indicated.

PROCEDURES FOR PERFORMING IN VIVO TUBERCULOSTATIC TESTS IN MICE

During the last few years, increasing use has been made of intravenously infected mice for the determination of the ability of substances to suppress experimental tuberculosis.

The use of mice for this purpose is favored by the following factors: an acute experiment of 3 to 4 weeks' duration can easily be performed; the intravenous route of inoculation by-passes intermediate barriers and delivers a nearly uniform dosage to the lungs; the variables of environment are readily controlled; statistically significant numbers of mice are more easily maintained than is true with larger animals; smaller amounts of drug are required; the histopathology has been studied and defined so the evaluation of results now may be more easily made. Furthermore, comparative studies have shown that the sulfonamides, sulfones, para-aminosalicylic acid, and streptomycin, substances which suppress experimental tuberculosis of guinea pigs, also have a similar action in mice, although the degree of suppression of the disease in mice is frequently less marked than in guinea pigs.

In the following pages will be detailed two methods which differ essentially in the nature of the test organism and in the method of evaluation of the results.

Method I

(42 to 49)

Mouse strain: Suitable strains of mice appear to be those which are either moderately resistant, such as the Strong "A" strain, or quite susceptible such as the CF1 strain. Young mice of these strains weighing 18 to 20 Gm. should be employed. At least 10 mice should be used for each treated and control group. These are infected intravenously with the desired inoculum of *M. tuberculosis* by injection in one of the lateral tail veins. With experience, 50 or 60 mice can be injected within an hour or less. The vein in the tail can be distended by the judicious use of heat or xylol. The inoculum can be contained in volumes of diluent of 0.2 to 0.5 cc. saline, 0.1 molar phosphate buffer, or the Proskauer and Beck synthetic medium may be used as a diluent for the suspension. When injecting mice, great care should be employed to avoid contamination of personnel. Gowns, masks, and rubber gloves are indicated and, following each injection, the tail of the mouse should be wiped off with a cotton swab soaked in 3.0 per cent cresol. Care must be taken also to ensure that each animal receives as nearly as possible the same number of tubercle bacilli. Since tubercle bacilli will tend to settle out or agglutinate spontaneously in the syringe, it is not advisable to fill a syringe and inject portions into successive animals. Rather, the syringe should be refilled each time from a suspension contained in a small

bottle fitted with a rubber vaccine bottle stopper which is shaken vigorously immediately prior to the withdrawal of each sample.

Intraperitoneal and intracerebral inoculation of tubercle bacilli have been employed, but insufficient data are available to recommend their use.

Test organism: Virulent human-type tubercle bacilli should be employed. The H37Rv strain is recommended. The suspensions used for inoculating the mice may be prepared and standardized by either of the two methods described for the preparation and standardization of inocula for the *in vitro* tests. The amount of inoculum to be employed should be 0.1 or 0.2 mg. wet weight of a 14- to 21-day-old surface culture, or a similar amount of a 7- to 10-day-old Dubos medium culture.

Administration of drugs: Depending upon their nature, drugs may be administered either orally or subcutaneously. The simplest method for the administration of drugs orally is the drug-diet method in which the drug is mixed with the diet and the mice allowed to partake of this ad libitum. It is necessary to administer some substances, such as streptomycin, subcutaneously in solution. The injections can be made most easily under the loose skin of the back.

Nature of experimental tuberculosis in mice: For a proper evaluation of the results of chemotherapeutic experiments against *M. tuberculosis* in mice using this method, a brief description of the disease process and the histopathologic picture in the lungs is necessary (45–47).

Following intravenous inoculation with the virulent H37Rv strain, mice appear essentially normal for approximately 14 days. There even may be some increase in weight during this period. Shortly after the first 2 weeks the majority of the animals will begin to lose weight, the fur will become ruffled, and the mice appear lethargic. As weight loss progresses, emaciation may become extreme. The majority of the animals will usually die within a period of 16 to 28 days. At autopsy gross lesions are usually seen only in the lungs. These consist of large whitish raised areas which frequently occupy 50 per cent or more of the lung substance. These "tubercles" consist of areas of necrosis (necrotic exudative lesions) which evolve in the following manner.

Following intravenous infection, the organisms are filtered out in the lungs and other organs. In the lungs, in response to the presence of the organisms, there is vascular congestion, diapedesis of some erythrocytes into the septa and alveoli, and proliferation of the macrophages from the resting interstitial mesenchyma, as well as their mobilization from the blood. This results in a diffuse septal thickening which is well developed within 4 days. As multiplication of the bacteria proceeds, the concomitant proliferation of the cellular elements becomes more extensive and more intensive, forming the focal "proliferative" lesions. These are small, but usually well defined, at 7 days following the infection. As the infecting agent continues to multiply, the cellular elements and other host tissues become necrotized. Neutrophil and other exudative elements respond to this development and the result is the typical "necrotic exudative" lesion swarming with bacteria and grossly tuberculoid. This type of change occurs rarely before 14 days, but usually predominates at 21 days.

Apparently the velocity of the evolution of the lesions from "proliferative type" to "necrotic exudative" determines the weight loss, survival time, and general appearance of the animal. Variations encountered between animals may be ascribed probably to variations in individual resistance of the different mice. Variations that may occur between different experiments may, in addition, have as their source variation in virulence between different cultures. Lesions in other organs than the lungs rarely progress beyond the proliferative stage.

With the above strain of *M. tuberculosis* and amount of inoculum, a small portion of the animals, which in some cases may be as high as 20 per cent, will not show the rapid fatal termination of the infection evidenced by the majority, but will all eventually die showing the pronounced involvement with necrotic exudative lesions.

Evaluation of results: During the course of chemotherapeutic experiments in mice, the animals should be weighed once a week and a record kept of the time of death and the weight at the time of death or sacrifice. At the end of three or four weeks, providing a majority of the untreated controls are dead, all of the remaining living mice should be sacrificed. At the time of death or sacrifice, each mouse should be autopsied, the lungs removed and fixed in 3.8 per cent formaldehyde. Following proper formaldehyde fixation, the lungs should be examined, and an estimate made of the amount of gross tuberculosis. This can be done on the basis of the following or some similar scheme:

0 —Apparently normal
1+—Less than 10 per cent of the organ replaced by gross pathologic changes
2+—10 to 25 per cent of the organ replaced by gross pathologic changes
3+—25 to 50 per cent of the organ replaced by gross pathologic changes
4+—Over 50 per cent of the organ replaced by gross pathologic changes

Following gross examination, the tissues should be dehydrated and paraffin-imbedded according to the usual technique, sections prepared and mounted, and sections from each mouse stained by the hematoxylin and eosin and by the Ziehl-Neelsen methods. These are then examined microscopically and an estimate made of the amount of tuberculosis tabulated on the following or some similar basis:

1+—A few discrete lesions involving less than 10 per cent of the organ
2+—Lesions involving 10 to 25 per cent of the organ
3+—Lesions involving 25 to 50 per cent of the organ
4+—Lesions involving over 50 per cent of the organ

These quantitative estimates should refer to the more or less finite lesions, rather than such general changes as congestion and edema. Sections stained for acid-fast bacilli and studied by oil immersion lens can be graded on the following or some similar basis:

0 —No acid-fast bacilli
1+—A few single or clumps of 4 or 5 intra- or extra-cellular organisms observed in occasional fields
2+—Moderate numbers of single organisms or groups of 4 or 5 organisms observed either intra- or extra-cellularly in about half of the fields
3+—Solitary bacilli and clumps of intra- and extra-cellular organisms observed in more than half of the fields
4+—Single organisms, clumps and large masses of organisms found within or outside of cells in more than half of the fields

In addition, a record should be made of the approximate relative predominance of proliferative and necrotic exudative lesions.

The evaluation of chemotherapeutic action will be made on the basis of the average survival time, the weight-response curve, and the histopathologic picture. The action of a highly effective chemotherapeutic agent such as streptomycin will be characterized in the mouse by either very few or no gross lesions, together with a prolonged survival time and a favorable weight response. The absence of gross lesions, however, cannot be taken as indicative of complete suppression of the infection, since microscopic examination may

reveal small proliferative lesions and numerous tubercle bacilli. A moderately effective chemotherapeutic agent will be characterized by rather extensive gross pulmonary changes composed of lesions which are microscopically almost entirely of the proliferative type, indicating a slower evolution of the disease. This is often associated with a prolonged survival time and frequently a favorable weight response, unless the agent is toxic. Very slight chemotherapeutic activity might be detected, in the absence of prolonged survival time or a favorable weight response, by the relative predominance of proliferative lesions as compared with the necrotic exudative lesions of the homologous control series. An ineffective chemotherapeutic agent will present the same picture as the controls or, if toxic, an exaggerated weight loss and curtailment of survival time.

The histopathologic examination also serves as an additional verification of the presence of tubercle bacilli in the tissues and may delineate intercurrent disease or toxic effect on the tissues.

Method II

(50 to 52)

This method takes advantage of the greater virulence for mice of *M. tuberculosis* var. bovis and the uniform degree of high susceptibility to infection with *M. tuberculosis* of the CF1 strain of homozygous white mice[1].

Mouse strain: Mice of the CF1 strain, either male or female, weighing between 16 and 18 Gm. and ranging in age from 4 to 6 weeks, will respond in a very similar fashion to a standard intravenous inoculation with the Ravenel bovine strain of *M. tuberculosis*. Mice older than 6 weeks of age should not be employed.

Test organism: The Ravenel strain of *M. tuberculosis* var. bovis has been found to be the most suitable. Care must be taken, however, to use rough virulent cultures of this strain. For the purpose of obtaining rough colony cultures to be used for mouse infection, a virulent culture of *M. tuberculosis* Ravenel is planted on Kirchner's agar containing 0.02 per cent Tween 80 and 0.25 per cent bovine serum albumin, Fraction V. A 5 per cent serum albumin solution made in distilled water and sterilized by filtration is added to the melted agar which has been cooled to 56° C. Four to six plates containing 15 ml. Kirchner's modified agar are inoculated with 0.1 ml. of a 10^{-4} dilution of culture. The inoculum is placed on the surface of the agar and allowed to spread by gently tilting the plates which are then sealed with cellulose tape and incubated at 37° C. for 21 days. Fresh cultures are started from rough colonies picked from these plates and such cultures, after two transfers in Kirchner's Tween-serum albumin medium, are injected into mice and then stored at 3° C. When found to be of satisfactory virulence they are available for use as seed cultures. These seed cultures will maintain their virulence when stored in the ice box in Kirchner's Tween 80-serum albumin medium at 3° C. for as long as six months.

Cultures to be used for mouse infections in the actual tests are inoculated with the seed cultures obtained as described above. These cultures which are grown in 50 ml. Erlenmeyer flasks which contain 20 ml. Kirchner's medium with 0.05 per cent Tween 80 and 0.1 per cent of human serum albumin are started by an inoculum of 2 ml. of the virulent stored culture and incubated for 5 days at 37° C. As a check on the purity of the culture, a transfer is made after 2 days' incubation to beef heart broth, a medium in which the tubercle bacillus does not grow, but in which usual contaminants grow well. As a further check on purity of culture, just before use films are made, stained by Ziehl-Neelsen method, and examined. Turbidity of the culture is then determined by comparison with

[1] May be obtained from Carworth Farms, New City, New York.

McFarland nephelometer tubes. With a standard procedure for maintaining cultures, the turbidity of Ravenel cultures is fairly constant. It has been observed, however, that when cultures lose virulence growth becomes heavier. The organisms of lesser virulence evidently reproduce more rapidly under the standard conditions described. If found to be of standard turbidity, the culture is diluted 1 to 10 with saline and injected intravenously into mice in 0.1 ml. amounts. This 1 to 10 dilution of the 5-day culture should contain on an average of 8 by 10^{-6} viable organisms as indicated by plate counts or the equivalent of approximately 0.0065 mg. of organisms (washed and dried weight). The synthetic Kirchner medium mentioned above has the following composition:

Na_2HPO_4	3.0 Gm.
KH_2PO_4	4.0 Gm.
$MgSO_4$	0.6 Gm.
Sodium citrate	2.5 Gm.
Asparagin	5.0 Gm.
Iron ammonium citrate	0.05 Gm.
Glycerol	20.0 ml.
Distilled water	1000.0 ml.

To this is added sufficient Tween 80 to give a concentration of 0.05 per cent. A 25 per cent solution of human serum albumin in distilled water sterilized by filtration is added to the previously autoclaved Kirchner Tween 80 medium in an amount sufficient to make a final concentration of 0.1 per cent serum albumin.

Method for conducting therapeutic tests: The mode of administration of drugs and the conduct of experiments are essentially the same as given in Method I.

Evaluation of results: When the CF1 mice are infected with the above standardized *M. tuberculosis* Ravenel inocula, the cumulative percentage dead plotted on a probability scale against time in days on an arithmetic scale gives a straight line. This indicates that the death-time relationship follows a normal frequency distribution curve[2]. This permits the calculation of the t_{50} (calculated 50 per cent mortality time) which should be used as the chief end-point criterion. Information is also obtained and recorded on the average survival time and the per cent survival for 35 days. With the above inoculum all of the control mice should die in from 16 to 36 days. Only 0.25 per cent can be expected to survive beyond 34 days. The favorable effect of any chemotherapeutic agent would be noted by a significant increase in the t_{50}. When using this method, autopsies are performed on the mice only for the purpose of certification as to cause of death. In the opinion of the originators of this method, nothing is learned by the subjective method of calculation (estimation of amount of tuberculous involvement upon autopsy) which is not given better by the other completely objective method of calculation (t_{50}). Such a conclusion, however, in no way argues against the obvious value of complete pathological examinations carried out to investigate the activity of any promising drug which emerges from the screening tests.

PROCEDURE FOR PERFORMING IN VIVO TUBERCULOSTATIC TESTS IN GUINEA PIGS

The guinea pig has long been used satisfactorily in experimental studies of tuberculosis. It is equally susceptible to human and bovine strains of bacilli, and with either type predictable results can be assured. Though certain strains of highly inbred guinea pigs

[2] A convenient type of graph paper for this purpose is the arithmetic probability chart sheet such as that printed by the Codex Book Co., Norwood, Massachusetts (Cat. No. 3127).

have been reported to be much more resistant to the disease than other inbred families (53), there is no great variation in the susceptibility of different guinea pig groups ordinarily obtained in the market. It is essential, however, that the animals used all be in good health. Guinea pigs over 250 Gm. in weight are suitable, although animals weighing 400 to 500 Gm. are preferable. It is imperative that animals of approximately the same weight be used in both the treated and control groups, with at least 10 animals in each.

Infection: The test organism can be introduced in a number of ways. The method ordinarily used is the subcutaneous injection in the groin. If a rapidly progressive disease is desired, 0.1 to 0.2 mg., wet weight, of virulent microorganisms is utilized. Within 21 days following such an inoculation, a subcutaneous abscess is present at the site of inoculation with enlarged draining inguinal nodes and visible tuberculous lesions in the various organs. A dosage of 0.001 mg., wet weight, of virulent bacilli usually produces a similar condition in 42 days. If the intracerebral route of infection is chosen, a small aperture is trephined in a dorsal area of the skull, and the bacilli are introduced into the brain by means of a tuberculin syringe with a one-quarter inch, 26-gauge needle (54). Five-hundredths of a cubic centimeter of a 6-day culture in Tween albumin liquid medium (55), containing 0.0001 mg., dry weight, of bacilli per cc. is injected.

Fixation and staining of tissues: The same procedures are used as for the mouse. Tissue from the site of inoculation, the draining lymph nodes, the lung, spleen, and liver should be examined for pathological changes and for microscopically visible tubercle bacilli. Cultures of the same tissues may be made to determine the number of viable bacilli present in a unit weight of each organ (56).

Pathology: The disease is manifested by a progressive inflammatory response in which there is at first a diffuse growth of macrophages, some of which are laden with tubercle bacilli (57). Later, the inflammation takes on a nodular character with the formation of epithelioid cells and the onset of caseation. The latter is ushered in by a polymorphonuclear infiltration. The bacilli multiply intensely in the first 2 weeks after infection. In the rapidly progressive disease the caseation is spotty and abounds in bacilli. Later, the caseous foci fuse and become amorphous. At this stage bacilli are few in the center of the caseous mass; they can be found in the transition zone between the living and the dead cells. With advanced disease large infarcts are commonly found in the liver and spleen. These should not be confused with true tuberculous foci, although the infarction is induced by strategically located tuberculous lesions. Occasionally the animal may die from hemorrhage due to rupture of a large infarcted spleen. Animals with extensive disease should be handled with care because of this possibility.

A more chronic type of tuberculosis is obtained in the guinea pig if the animals are first vaccinated with killed, or with living, avirulent bacilli (58, 59). A lapse of about 6 weeks to 2 months should be allowed between vaccination and reinfection with virulent microorganisms. The same methods of introducing the virulent bacilli can be used as was employed in normal animals.

Recording of results: Animals should be weighed as suggested above in the mouse technique, and clinical observations should be made at the same time concerning the appearance or disappearance of the draining lymph nodes, the growth and healing of the ulcer at the site of inoculation, and the changes in the gait and general appearance of the animals.

The same method of recording results used for mice can be adapted to the guinea pig. For purposes of comparison, the extent of the involvement in the spleen, liver, lungs, and lymph nodes can be noted, and numerical values, proportionate to the extent and severity

of the disease, can be assigned to the tuberculosis in each organ (59). The maximum rating of 4 for any organ indicates grossly visible, widespread, caseous tuberculosis. The maximum value of 16 for the tuberculosis in the animal as a whole denotes an advanced generalized disease. One may also record the results in the following manner: A schematic representation of the various organs is printed on the record card of each animal (54) by means of a large rubber stamp measuring approximately 2 by 4 inches (5 by 10 cm.). At the time of necropsy, the observer records the grossly noted lesions by sketching in the approximate shape and size of the tuberculous foci with a red pencil in each of the organs involved. If the lesions are too extensive to be recorded individually, the whole organ is colored red. Appropriate marginal notes can be made at this time to indicate the size and appearance of the organs.

PROCEDURE FOR PERFORMING IN VIVO TUBERCULOSTATIC TESTS IN RABBITS

The rabbit can be used to advantage if virulent bovine or avian type of tubercle bacilli are employed as the infectious agent in chemotherapeutic studies, and if predictable progressive and fatal disease is desired. The great varying natural resistance of the market rabbit to the virulent human-type bacillus precludes its use if strictly predictable results are envisaged (61). It would seem advisable, however, to collect data relative to the effect of various chemotherapeutic agents on the tuberculous disease caused by the human bacillus in highly inbred rabbits of low genetic resistance to the infection. It has been recently demonstrated that naturally resistant, inbred rabbits completely overcome an infection induced by the quantitative inhalation of virulent human-type tubercle bacilli which causes a progressive disease in genetically susceptible animals simultaneously exposed to the same inhalation (62). It has been found in this study that resistant rabbits markedly inhibit the multiplication of virulent human-type tubercle bacilli in the macrophages of the lung and soon destroy them altogether, whereas the same bacilli multiply abundantly in the lung of susceptible rabbits for a long time. The implication of these results for the *in vivo* studies of bactericidal and bacteriostatic agents in tuberculosis is obvious.

If the virulent avian type of tubercle bacillus is used, intravenous injection of 0.1 to 0.001 mg., wet weight, of microorganisms will cause progressive disease and death frequently within 3 weeks. The liver, spleen, and bone marrow are the loci of the infection. In this Yersin type of disease, the inflammatory reaction consists almost wholly of a diffuse infiltration of macrophages with very little, if any, necrosis. Bacilli are always numerous in these lesions after about 2 weeks (63, 64).

If virulent bovine tubercle bacilli are used, the bacilli may be injected intravenously, subcutaneously, or they may be inhaled through small droplets suspended in the air.

All routes of infection will produce extensive pulmonary tuberculosis eventually. However, the most predictable results can be obtained from inhalation of contaminated air or from intravenous injection. The simplest and easiest way to produce predictable, rapidly progressive, and fatal pulmonary disease is to inject 0.1 to 0.001 mg., wet weight, of bacilli intravenously. By this method the animals will all die in 6 weeks to 6 months, depending on the dosage of bacilli, with extensive pulmonary disease, and with lesions of minor extent in other organs.

If a more chronic, progressive pulmonary tuberculosis is desired, infection through the inhaled air is the method of choice (65). This procedure requires a special inhalation chamber and a rather complicated technique, and should not be attempted unless proper laboratory equipment is available. However, it should become the method of choice, since, by proper control, one can consistently induce a disease in rabbits which closely

simulates the origin and progress of chronic ulcerative pulmonary phthisis as seen in man.

The inhalation method not only offers an opportunity of studying the effects of therapeutic agents on the progression and healing of cavities, but a means is also afforded of elucidating the emergence of strains of tubercle bacilli which are resistant to a given therapeutic agent. It has been demonstrated by a number of observers that streptomycin-resistant strains are found much more commonly in human pulmonary tuberculosis with cavitation than in pulmonary disease without excavation (66).

Pathology: The pathology of bovine tuberculosis is the same regardless of the route of infection or the dosage of bacilli. However, the phenomenon of caseation becomes a major part of the disease in animals which have survived several months. In the first few days the predominant cell is the neutrophil which is soon replaced by macrophages (monocytes). Bacilli are few in number at first, but they become numerous in the foci within two weeks. Necrosis of the inflammatory exudate and of the fixed tissue seldom is evidenced prior to 2 weeks and is much more pronounced in animals which survive 6 weeks or longer (67). If the animals survive 3 months or longer, softening of the caseous lesions often takes place and cavity formation is common in animals that live longer than 6 months. Bacilli become less numerous during the process of necrosis. During liquification of the necrotic lesions and the process of cavity formation, however, the bacilli may be found in large numbers in the inner portions of the cavity (68).

The pathologic picture of chronic tuberculosis in the lungs of rabbits infected with virulent bovine tubercle bacilli resembles so closely that commonly encountered in chronic pulmonary tuberculosis in man that it seems desirable to study the effect of chemotherapeutic agents on this type of experimentally produced tuberculosis. Any chemotherapeutic agent, or any combination of such agents, that will so favorably affect chronic ulcerative pulmonary tuberculosis in the rabbit that a complete healing can occur may well afford a clue relative to a successful attack on chronic pulmonary tuberculosis in man. It is the caseous, cavitating phase of tuberculosis that so far has not been decisively affected by any chemotherapeutic procedure.

An even more chronic pulmonary tuberculosis can be produced in rabbits by vaccination with 10 mg. living, avirulent bacilli (BCG or RI or H37Ra) subcutaneously or intravenously. In this procedure the animals should be maintained for 6 weeks to 2 months after vaccination before they are given a virulent infection, either by inhalation or by intravenous infection. Dosages similar to those used in nonvaccinated animals should be employed in the reinfection.

Recording of results: The same procedure for recording results may be used for rabbits as for mice and guinea pigs.

Specimens of tissue from lungs, kidney, spleen, and intestine are usually sufficient for microscopic study. These specimens are treated in the same way as those from the other species. The phase of tuberculosis that should receive special attention is any evidence of healing of the caseous and ulcerative tuberculous foci, particularly in the lungs.

COMBINED THERAPY

(69 to 73)

It is occasionally of value to determine the effect of two or more agents administered simultaneously on experimental tuberculosis of guinea pigs or mice. The principles and methods involved in such experiments are identical with those already given. Care must be taken, however, to include groups of animals treated with each agent alone to serve as a basis for the determination of whether an additive or synergistic effect has been obtained

with the agents employed together. Each substance should be administered in a dosage in which it has of itself some chemotherapeutic effect (74). In addition, the amount of each substance administered must be small enough so that it alone will not give a maximal therapeutic effect. Unless this is done, it will be impossible to determine whether an increased therapeutic action has taken place as a result of the combined therapy.

A Method for the Determination of the Rate of Growth of Tubercle Bacilli by the Use of Small Inocula (77)

Inocula containing different quantities of tubercle bacilli can be used to estimate the rate of growth of tubercle bacilli, provided the rate of growth is the same regardless of the number of bacilli used to inoculate the tubes, and provided a method can be devised for

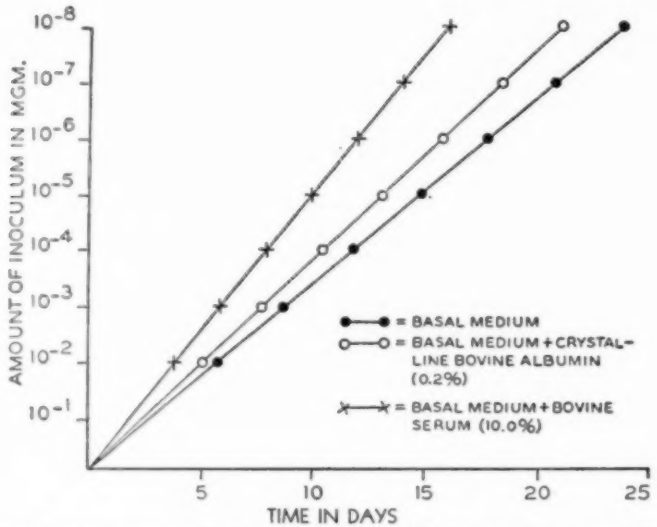


Fig. 1. The rate of growth of *M. Tuberculosis* (H37Rv) in three types of media.

This figure is reproduced through the courtesy of the Journal of Bacteriology, published by Williams & Wilkins Company, Baltimore, Md., 1949, 57, 247.

determining the time at which each inoculum grows up to a certain standard mass. The latter can be accomplished by recording the time at which growth with each inoculum first becomes visible. If the rates of growth of the inocula employed are the same, a linear relationship should be obtained when the time of first appearance of growth of each inoculum is plotted against the logarithm of the amount of inoculum employed. The slope of the straight line so obtained will represent the rate of growth of the tubercle bacilli and can be used for the calculation of the growth rate and, if desired, the generation time. Thus, relatively accurate comparisons could be made of the rate of growth of tubercle bacilli in different media and under different conditions.

The practicability of this method for the estimation of the rate of growth of tubercle bacilli has been tested in the following manner. Suspension of 14- to 21-day-old cultures of the H37Rv stain of *M. tuberculosis* var. hominis were prepared and standardized (78). From this suspension dilutions were made with 0.01 M phosphate buffer solution, pH

7.0, so that the inocula to be employed contained, respectively, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} mg. tubercle bacilli wet weight. Dilutions can also be prepared directly from cultures grown in Tween albumin medium. Ten tubes, each containing 5.0 ml. of the modified Proskauer and Beck basal synthetic medium (79), were inoculated with each of the 8 quantities of H37Rv, making a total of 80 tubes in all. In addition, two modifications of the basal medium containing, respectively, 0.2 per cent crystalline bovine albumin and 10.0 per cent sterile beef serum, were inoculated in the same manner. All tubes were then incubated at 37° C., and examined daily for the presence of the typical granular growth. The time of the first appearance of such growth for each inoculum in each medium was recorded and then plotted against the logarithm of the inoculum. The results are shown in figure 1. The actual quantities of tubercle bacilli may be used along the ordinate since they are proportional to the logarithms.

TABLE 1

Time in Days of First Appearance of Growth of Mycobacterium Tuberculosis (H37Rv) in Three Types of Liquid Media

Amount of Inoculum	Medium																	
	Basal Medium						Basal Medium with Crystalline Bovine Albumin (0.2%)						Basal Medium with Beef Plasma (10.0%)					
	Experiment Number						Experiment Number						Experiment Number					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Mg.																		
10^{-1}		2	2	2	2	2		2	2	2	2	2		2	2	2	2	2
10^{-2}	6	5	5	5	4	5	5	4	5	5	4	4	4	4	4	4	4	4
10^{-3}	9	8	8	8	7	8	8	7	7	7	7	7	6	6	6	6	6	6
10^{-4}	12	11	11	10	10	10	10	9	9	9	9	9	8	8	8	8	8	8
10^{-5}	15	14	14	13	13	13	13	12	12	12	11	12	10	10	10	10	10	10
10^{-6}	18	16	17	16	16	16	16	14	14	14	14	14	12	12	12	12	12	12
10^{-7}	21	19	20	19	19	19	19	16	17	16	16	16	14	14	14	14	14	14
10^{-8}	24						21					18	16	16	16	16	16	16

This table is reproduced through the courtesy of the Journal of Bacteriology, published by Williams & Wilkins Company, Baltimore, Md., 1949, *57*, 247.

An examination of figure 1 reveals that a linear relationship does exist between the time of the first appearance of growth and the logarithms of the number of organisms used as inocula. This indicates that the rate of growth is the same regardless of the amount of inoculum employed. Furthermore, the slopes of the lines in figure 1 represent the actual rates of growth. In such experiments the lack of a linear relationship between the time of appearance of growth and the logarithm of the inoculum would indicate either technical errors or actual differences in the rates of growth of the various inocula.

From the data in figure 1 the slope of the line and therefore the growth rate were calculated by use of the following formula:

$$K = \frac{\log a - \log b}{t}$$

Where K = growth rate constant
a = largest inoculum employed (in mg.)
b = smallest inoculum employed
t = time in days or hours

The generation time (time for one cell division) was calculated in the following manner:

$$G = \frac{\log 2}{K}$$

Where G = generation time
K = growth rate constant

A total of six experiments were done at different times similar to the one represented by figure 1. Table 1 gives the findings in detail of these six experiments, and figure 1 shows graphically the results obtained in one such experiment. The actual growth rate constants and generation times obtained in each experiment are given in table 2. The

TABLE 2

The Rate of Growth of the Virulent Human-type Tubercle Bacillus (H37Rv) in Three Types of Media

EXPERIMENT NUMBER	MEDIUM					
	Basal Medium		Basal Medium plus Crystalline Bovine Albumin (0.2%)		Basal Medium plus Beef Plasma (10.0%)	
	Growth Rate Constant (K)	Generation Time in Hours	Growth Rate Constant (K)	Generation Time in Hours	Growth Rate Constant (K)	Generation Time in Hours
1	0.333	21.7	0.375	19.3	0.5	14.4
2	0.375	19.3	0.428	16.9	0.5	14.4
3	0.375	19.3	0.428	16.9	0.5	14.4
4	0.352	20.5	0.428	16.9	0.5	14.4
5	0.375	19.3	0.428	16.9	0.5	14.4
6	0.375	19.3	0.428	16.9	0.5	14.4
Mean	0.364	19.90	0.419	17.3	0.5	14.4
Standard Deviation		±1.0		±0.96		0.0
			P = <0.01			

This table is reproduced through the courtesy of the Journal of Bacteriology, published by Williams & Wilkins Company, Baltimore, Md., 1949, *57*, 247.

high degree of reproducibility of results using this method is apparent, and the high degree of significance of the difference in growth rate between the basal medium and that medium containing crystalline bovine albumin indicates its usefulness. The tubercle bacilli actually grew on an average 13 per cent faster in the medium containing the bovine albumin. The marked stimulating effect of bovine plasma on the growth of tubercle bacilli is obvious, the average increase in rate over the basal medium being 27.6 per cent.

The technique as carried out in the experiments mentioned above is still laborious, involving as it does the preparation of 80 tubes of medium and the repeated handling of large numbers of tubes. This can be simplified considerably by employing fever inocula. Actually, only two inocula would be necessary in order to establish the slope of the line and permit a calculation of the growth rate. For the sake of accuracy three are preferred: either 10^{-3}, 10^{-4}, and 10^{-5}, or 10^{-3}, 10^{-4}, and 10^{-5} mg. of tubercle bacilli wet weight. The number of tubes inoculated with each quantity of tubercle bacilli can be reduced, since sampling errors are not significant with amounts of 10^{-4} mg. or more. Five tubes

are now used routinely since this number usually prevents technical errors or contamination from invalidating an experiment. This reduces the number of tubes for a growth rate estimation from 80 to only 15. The tubes should be examined every day, however, or at the least every other day. If readings are made at a longer interval, gross errors are introduced.

The technique of examining the tubes for the presence of growth is important. Tubes of uninoculated medium should be used for comparative purposes. A constant source of light and a dark background are essential. The use of a Quebec Colony Counter is ideal. This not only provides a constant light source and a dark background, but a small amount of magnification which facilitates the readings.

With practice, the error is not more than plus or minus one day. Some objective method for the detection of a standard number of tubercle bacilli would be highly desirable, but, because of the flocculent type of growth, this is not practicable at present.

REFERENCES

(1) White, H. J., Bratton, A. C., Litchfield, J. T., and Marshall, E. K., Jr.: The relationship between the *in vitro* and the *in vivo* activity of sulfonamide compounds, J. Pharmacol. & Exper. Therap., 1941, *72*, 112.

(2) Youmans, G. P., Feldman, W. H., and Doub, L.: A comparison of the effect of *p*-amino phenyl sulfone compounds *in vitro* and *in vivo* on tubercle bacilli, Am. Rev. Tuberc., 1946, *54*, 295.

(3) Smith, M. I., Emmart, E. W., and Westfall, B. B.: The action of certain sulfonamides, sulfones, and related phosphorus compounds in experimental tuberculosis, J. Pharmacol. & Exper. Therap., 1942, *74*, 163.

(4) Boissevain, C. H.: Effect of sulfanilamide and napthoquinone derivatives on the growth of the tubercle bacillus, Tr. Nat. Tuberc. A., 1940, *56*, 88.

(5) Ballon, H. C., and Guernon, A.: The action of sulfanilamide upon the growth of the tubercle bacillus *in vitro*, J. Thoracic Surg., 1938, *8*, 184.

(6) Ballon, H. C., and Guernon, A.: The effect of sulfanilamide upon the growth of acid-fast bacilli *in vitro*, Tubercle, 1940, *21*, 153.

(7) Birkhaug, K. E.: Bacteriostatic effect of sulfapyridine, sulfanilamide and prontosil rubrum *in vitro* on mycobacteria, Proc. Soc. Exper. Biol. & Med., 1939, *42*, 275.

(8) Follis, R. H.: Sulfanilamide, acetylsulfanilamide and sulfapyridine: Their effect on mycobacterium tuberculosis hominis *in vitro*, Am. Rev. Tuberc., 1940, *41*, 117.

(9) Heise, F. H., and Steenken, W.: Growth and virulence of tubercle bacilli: A study of the effect of vitamins B_1 and B_2, Riboflavin, colchicine, phthiocol and thyloquinone, Am. Rev. Tuberc., 1941, *44*, 635.

(10) Rist, N. C. R.: Action du *p*-aminophenylsulfamide et de la *p*-diaminodiphenylsulfone sur la culture des bacilles tuberculeux des mammiferes et des oiseaux, Soc. de biol., 1939, *130*, 972.

(11) Middlebrook, G., and Lloyd, J. B.: Effects of pH, temperature and salicylate on bacteriostasis of tubercle bacilli by sulfonamides and diaminodiphenylsulfone, Am. Rev. Tuberc., 1944, *49*, 535.

(12) Middlebrook, G., and Lloyd, J. B.: Bacteriostatic activity of some new derivatives of diaminodiphenylsulfone and naphthoquinones against the tubercle bacillus, Am. Rev. Tuberc., 1944, *49*, 539.

(13) Saz, A. K., and Bernheim, F.: The effect of 2, 3, 5-Triiodobenzoate and various other compounds on the growth of the tubercle bacillus, J. Pharmacol. and Exper. Therap., 1941, *73*, 78.

(14) Cutting, W. C., Beghardt, L. P., Proescher, F., and Durrum, E.: Chemotherapy in experimental tuberculosis: Tests *in vitro* and *in vivo* with different types of agents, Am. Rev. Tuberc., 1945, *52*, 73.

(15) Drea, W. F.: The growth of human tubercle bacilli, H37, in synthetic medium with and without agar, J. Bact., 1940, *39*, 197.

(16) Drea, W. F.: Growth of small numbers of tubercle bacilli, H37, in Long's liquid synthetic medium and some interfering factors, J. Bact., 1942, *44*, 149.

(17) Drea, W. F.: Depth growth of acid-fast bacilli in liquid media: I. Technique, Am. Rev. Tuberc., 1946, *53*, 353.

(18) Drea, W. F.: Depth growth of acid-fast bacilli in liquid media: II. Study of various technical and theoretical aspects, Am. Rev. Tuberc., 1946, *53*, 363.

(19) Youmans, G. P.: Subsurface growth of virulent human tubercle bacilli in a synthetic medium, Proc. Soc. Exper. Biol. & Med., 1944, *57*, 122.

(20) Youmans, G. P.: An improved method for testing of bacteriostatic agents using virulent human type tubercle bacilli, Proc. Soc. Exper. Biol. & Med., 1944, *57*, 119.

(21) Youmans, G. P., and Karlson, A. G.: Streptomycin sensitivity of tubercle bacilli: Studies on recently isolated tubercle bacilli and the development of resistance to streptomycin *in vivo*, Am. Rev. Tuberc., 1947, *55*, 529.

(22) Youmans, G. P.: The effect of para-aminosalicylic acid *in vitro* and *in vivo* on virulent human type tubercle bacilli, Quart. Bull., Northwestern Univ. Med. School, 1946, *20*, 420.

(23) Youmans, G. P., Raleigh, G. W., and Youmans, A. S.: The tuberculostatic action of para-aminosalicylic acid, J. Bact., 1947, *54*, 409.

(24) Youmans, G. P., Youmans, A. S., and Osborne, R. R.: Tuberculostatic action of chloromycetin *in vitro* and *in vivo*, Proc. Soc. Exper. Biol. & Med., 1948, *67*, 426.

(25) Steenken, W., Jr., and Heise, F. H.: Action of promin and diaminodiphenyl sulfone upon tubercle bacilli, Proc. Soc. Exper. Biol. & Med., 1943, *52*, 180.

(26) Wolinsky, E., and Steenken, W., Jr.: Effect of streptomycin on the tubercle bacillus: The use of Dubos' and other media in tests for streptomycin sensitivity, Am. Rev. Tuberc., 1947, *55*, 281.

(27) Wolinsky, E., Reginster, A., and Steenken, W., Jr.: Drug resistant tubercle bacilli in patients under treatment with streptomycin, Am. Rev. Tuberc., 1948, *58*, 335.

(28) Wolinsky, E., Wetzel, V., and Steenken, W., Jr.: Tuberculostatic effect of furacin *in vitro* and *in vivo*, Proc. Soc. Exper. Biol. & Med., 1949, *70*, 483.

(29) Steenken, W., Jr., and Wolinsky, E.: Tuberculostatic activity of aureomycin *in vitro* and *in vivo*, Am. Rev. Tuberc., 1949, *59*, 221.

(30) Steenken, W., Jr., and Wolinsky, E.: The tuberculostatic effect of subtilin *in vitro* and *in vivo*, J. Bact., 1949, *57*, 453.

(31) Dubos, R. J.: Effect of lipids and serum albumin on bacterial growth, J. Exper. Med., 1947, *85*, 9.

(32) Kirby, W. M. M., and Dubos, R. J.: Effect of penicillin on the tubercle bacillus *in vitro*, Proc. Soc. Exper. Biol. & Med., 1947, *66*, 120.

(33) Fisher, M. W.: Sensitivity of tubercle bacilli to streptomycin: An *in vitro* study of some factors affecting results in various test media, Am. Rev. Tuberc., 1948, *57*, 58.

(34) Anderson, H. H., and Wong, S. C.: Antibiotics in experimental tuberculosis (with special reference to subtilin), Tuberculology, 1946, *8*, 77.

(35) Anderson, H. H.: New surface active antibiotics, J. Invest. Dermat., 1947, *8*, 25.

(36) Bloch, H., Erlenmeyer, H., and Suter, E.: Concerning *in vitro* assay of growth inhibitors for tubercle bacilli, Experientia, 1947, *3*, 199.

(37) Forrest, H. S., Hart, P. D'A., and Walker, J.: Antagonistic effect by certain growth-stimulants of the tuberculostatic activity of 5-amino-2-butoxypyridine, Nature, 1947, *160*, 94.

(38) Youmans, A. S., and Youmans, G. P.: The effect of Tween 80 *in vitro* on the bacteriostatic activity of twenty compounds for *Mycobacterium tuberculosis*, J. Bact., 1948, *56*, 245.

(39) Williston, E. H., Zia-Walrath, P., and Youmans, G. P.: Plate methods for testing antibiotic activity of actinomycetes against virulent human type tubercle bacilli, J. Bact., 1947, *54*, 563.

(40) Tobie, W. C.: *Mycobacterium tuberculosis* no. 607 and similar doubtful tubercle bacilli: A review, Am. Rev. Tuberc., 1948, *58*, 693.

(41) Youmans, G. P.: The effect of streptomycin *in vitro* on *M. tuberculosis* var. hominis, Quart. Bull., Northwestern Univ. Med. School, 1945, *19*, 207.

(42) Youmans, G. P., and McCarter, J. C.: A preliminary note on the effect of streptomycin on experimental tuberculosis of white mice, Quart. Bull., Northwestern Univ. Med. School, 1945, *19*, 210.

(43) Youmans, G. P., and McCarter, J. C.: Streptomycin in experimental tuberculosis: Its effect on tuberculous infections in mice produced by *M. tuberculosis* var. hominis, Am. Rev. Tuberc., 1945, *52*, 432.

(44) Youmans, G. P., and Williston, E. H.: Effect of streptomycin on experimental infections produced in mice with streptomycin resistant strains of *M. tuberculosis* var. hominis, Proc. Soc. Exper. Biol. & Med., 1946, *63*, 131.

(45) Raleigh, G. W., and Youmans, G. P.: The use of mice in experimental chemotherapy of tuberculosis: I. Rationale and review of the literature, J. Infect. Dis., 1948, *82*, 197.

(46) Raleigh, G. W., and Youmans, G. P.: The use of mice in experimental chemotherapy of tuberculosis: II. Pathology and pathogenesis, J. Infect. Dis., 1948, *82*, 205.

(47) Youmans, G. P., and Raleigh, G. W.: The use of mice in experimental chemotherapy of tuberculosis: III. The histopathologic assay of chemotherapeutic action, J. Infect. Dis., 1948, *82*, 221.

(48) McKenzie, D., Malone, L., Kushner, S., Oleson, J. J., and Subbarow, Y.: The effect of nicotinic acid amide on experimental tuberculosis of white mice, J. Lab. & Clin. Med., 1948, *33*, 1249.

(49) Chin, Y. C., Anderson, H. H., Alderton, G., and Lewis, J. C.: The antituberculous activity and toxicity of lupolin for the mouse, Proc. Soc. Exper. Biol. & Med., 1949, *70*, 158.

(50) McKee, C. M., Rake, G., Donovick, R., and Jambor, W. P.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: I. Choice and standardization of culture, Am. Rev. Tuberc., 1949, *60*, 90.

(51) Donovick, R., McKee, C. M., Jambor, W. P., and Rake, G.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: II. Choice of mouse strain, Am. Rev. Tuberc., 1949, *60*, 109.

(52) Rake, G., Jambor, W. P., McKee, C. M., Pansy, F., Wiselogle, F. Y., and Donovick, R.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: III. The standardized test, Am. Rev. Tuberc., 1949, *60*, 121.

(53) Wright, S., and Lewis, P. A.: Factors in the resistance of guinea pigs to tuberculosis, with especial regard to inbreeding and heredity, Am. Naturalist, Lancaster, Pa., 1921, *55*, 20.

(54) Smithburn, K. C.: Resistance to tuberculosis: Factors associated with bacteria, Am. Rev. Tuberc., 1939, *39*, 371.

(55) Dubos, R. J., and Davis, B. D.: Factors affecting growth of tubercle bacilli in liquid media, J. Exper. Med., 1946, *83*, 409.

(56) Lurie, M. B.: Fate of B.C.G. and associated changes in organs of rabbits, J. Exper. Med., 1934, *60*, 163.

(57) Woodruff, C. E., and Kelly, R. G.: Growth of tubercle bacilli in tissues of normal and allergic guinea pigs, Am. Rev. Tuberc., 1940, *42*, 782.

(58) Baldwin, E. R., and Gardner, L. U.: Reinfection in tuberculosis: Experimental arrested tuberculosis and subsequent infections, Am. Rev. Tuberc., 1921, *5*, 429.

(59) Steenken, W., Heise, F. H., and Wolinsky, E.: Treatment of experimental tuberculosis in vaccinated and non-vaccinated guinea pigs with promin, Am. Rev. Tuberc., 1943, *48*, 453.

(60) Feldman, W. H., Hinshaw, H. C., and Mann, F. C.: Streptomycin in experimental tuberculosis, Am. Rev. Tuberc., 1945, *52*, 269.

(61) Heppleston, A. G.: Quantitative air-borne tuberculosis in the rabbit: The course of human-type infection, J. Exper. Med., 1949, *89*, 597.

(62) Lurie, M. B., Abramson, S., and Heppleston, A. G.: Varying genetic resistance of rabbits to quantitative inhalation of human tubercle bacilli, Fed. Proc., 1949, *8*, 361.

(63) Griffith, A. S.: A System of Bacteriology, London, 1930, *5*.

(64) Lurie, M. B.: Symposium Series, Am. Assoc. for the Advancement of Science, 1938, *1*, 25.

(65) Lurie, M. B., and Abramson, S.: Reproduction of human ulcerative pulmonary tuberculosis in rabbits by quantitative natural airborne contagion, Proc. Soc. Exper. Biol. & Med., 1948, *69*, 531.

(66) Howard, W. L., Maresh, F., Mueller, E. E., Yannitelli, S. A., and Woodruff, C. E.: The role of pulmonary cavitation in the development of bacterial resistance to streptomycin, Am. Rev. Tuberc., 1949, *59*, 391.

(67) Lurie, M. B.: Correlation between histological changes and fate of living tubercle bacilli in organs of tuberculous rabbits, J. Exper. Med., 1932, *55*, 31.

(68) Lurie, M. B.: Correlation between histological changes and fate of living tubercle bacilli in organs of reinfected rabbits, J. Exper. Med., 1933, *57*, 181.

(69) Youmans, G. P., Youmans, A. S., and Osborne, R. R.: The combined effect of streptomycin and para-aminosalicylic acid on experimental tuberculosis in mice, Journal-Lancet, 1947, *67*, 403.

(70) Smith, M. I., and McCloskey, W. T.: The chemotherapeutic action of streptomycin and promin in experimental tuberculosis, Pub. Health Rep., 1945, *60*, 1129.

(71) Smith, M. I., McCloskey, W. T., and Emmart, E. W.: Influence of streptomycin and promin on proliferation of tubercle bacilli in the tissues of albino rat, Proc. Soc. Exper. Biol. & Med., 1946, *62*, 157.

(72) Smith, M. I., McCloskey, W. T., Jackson, E. L., and Bauer, H:. Chemotherapeutic action of streptomycin and of streptomycin with a sulfone or sulfadiazine on tuberculosis, Proc. Soc. Exper. Biol. & Med., 1947, *64*, 261.

(73) Smith, M. I., McCloskey, W. T., and Jackson, E. L.: Studies on chemotherapy of tuberculosis: VIII. The comparative action of four sulfones in experimental tuberculosis in guinea pigs and the combined action of streptomycin with one of the sulfones, Am. Rev. Tuberc., 1947, *55*, 366.

(74) Klein, M., and Kimmelman, L. J.: The correlation between the inhibition of drug resistance and synergism in streptomycin and penicillin, J. Bact., 1947, *54*, 363.

(75) Howlett, K. S., Jr., O'Connor, J. B., Sadusk, J. F., Jr., Swift, W. E., Jr., and Beardsley, F. A.: Sensitivity of tubercle bacilli to streptomycin: The influence of various factors upon the emergence of resistant strains, Am. Rev. Tuberc., 1949, *59*, 402.

(76) Medlar, E. M., and Sasano, K. T.: Effects of virulence of microorganisms on histopathology of experimental tuberculosis as observed in normal rabbits injected intravenously with tubercle bacilli of high and of low virulence, Am. Rev. Tuberc., 1933, *28*, 62.

(77) Youmans, G. P., and Youmans, A. S.: A method for the determination of the rate of growth of tubercle bacilli by the use of small inocula, J. Bact., 1949, *58*, 247.

(78) Youmans, G. P., and Karlson, A. G.: Streptomycin sensitivity of tubercle bacilli: Studies on recently isolated tubercle bacilli and the development of resistance to streptomycin *in vivo*, Am. Rev. Tuberc., 1947, *55*, 529.

(79) Youmans, G. P.: A method for the determination of the culture cycle and the growth rate of virulent human type tubercle bacilli, J. Bact., 1946, *51*, 703.

AMERICAN TRUDEAU SOCIETY

The James Alexander Miller Fellowship for Research in Tuberculosis

The New York Tuberculosis and Health Association announces that a fellowship will be available from July 1, 1950 to June 30, 1951.

The fellowship is designed to support a qualified medical investigator who will devote full time to a research project with a definite bearing on tuberculosis. Assurance must be provided that the applicant will be acceptable in the laboratory or clinic of his choice and that he will be provided with the facilities necessary for the pursuit of the work. It is preferred that such laboratory or clinic be located in New York City.

The stipend will be $5,000.

Application forms will be supplied on request to the New York Tuberculosis and Health Association, 386 Fourth Avenue, New York 16, N. Y., and must be submitted in duplicate not later than March 1, 1950. Announcement of the award will be made in April 1950.

THE AMERICAN REVIEW OF TUBERCULOSIS ABSTRACTS

Edited by LAWRENCE B. HOBSON

VOLUME 61 FEBRUARY, 1949 ABST. NO. 2

CLINICAL STUDIES, TUBERCULOSIS

Erythema Nodosum.—It is now generally agreed that erythema nodosum is not a specific disease but rather a nonspecific reaction to various infections and toxic agents. It occurs most commonly in association with primary tuberculous infection although sensitivity to sulfonamide occasionally produces erythema nodosum, as does coccidioidomycosis. In a series of 124 cases there were twice as many female patients as male. Roentgenograms revealed pulmonary changes in 56 patients, 49 having a primary tuberculous infection, 2 having pleural effusion, probably tuberculous, 4 having a reinfection type of tuberculosis. One case was not diagnosed definitely. The remaining 68 patients had negative chest roentgenograms. Of these, 44 had positive tuberculin skin tests and 24 had negative tuberculin reactions. Thus, of the 124 cases, 99 were regarded as probably tuberculous. Of the 99, 71 probably were cases of primary tuberculous infection.—*Erythema nodosum, J. H. Middlemiss, Brit. J. Radiol., July, 1949, 22: 375. (B. Hyde)*

Obstetrical Tuberculosis.—Smears, cultures, and guinea pig inoculation were used in 92 tuberculous obstetrical patients in order to recover tubercle bacilli from the placenta, breast milk, and umbilical cord blood and from the lungs, liver, and spleen of dead infants. Of 92 placentae from mothers with active pulmonary tuberculosis, 11 (11.9 per cent) proved positive. None of 67 placentae from patients with inactive types of the disease contained bacilli. Of 147 blood specimens, from the umbilical cord, 4 (2.7 per cent) were positive and 2 (2.1 per cent) of 96 breast milk samples contained bacilli. A special study was made of 39 cases of premature birth in tuberculous mothers, of whom 21 (51 per cent) died, 17 of them within the first month after delivery. Of their 39 babies, 33 (84.6 per cent) died within the first month and the remaining 6, within six months. Congenital tuberculosis was the cause of death in 5 infants. The presence of tuberculous lesions in the placenta or of tubercle bacilli in the umbilical blood or amniotic fluid does not necessarily mean fetal infection. Likewise, bacilli may be present in the placenta or the mammary gland in the absence of lesions in these organs.—*Tuberculose e gravidez e tuberculose congênita: Aspecto bacteriológico do problema, M. Fontes-Magarão, H. Linhares, & A. Vieira, Clin. Tisiol., October-December, 1947, 2: 433.—(A. A. Moll)*

Tuberculous Empyema.—From 1941 to 1947, 74 cases of tuberculous empyema were treated in Norwegian hospitals. In 19, the patient's condition did not allow major surgery. In 55, a plastic operation was performed on the chest wall. In 19 cases, no tubercle bacilli were found in the pleural fluid. In 12 of these, secondary infection was present. As of May 1948, 25 were dead. Of the 49 survivors, 43 had been followed. When an adequate thoracoplasty was carried out, satisfactory results were permanent, and no patient had a symptomatic recurrence of tuberculous activity in the lung on the side of the thoracoplasty. Two died of contralateral tuberculosis, and 2 had active disease in the contralateral lung and other organs. A total of 28 patients were considered "cured" and allowed to carry on nor-

mal occupations without heavy exertion. A group of 6 considered "improved" included those with fistula after the operation or with roentgenographic evidence of residual fluid. Prognosis in this group is good and all were at work. Two patients were "unimproved," one with active tuberculosis of the contralateral lung, the other with contralateral, spinal, and renal disease. The empyema arose spontaneously in 20 patients, 90 per cent of whom had a mixed infection. In the remaining 54 patients, empyema was a complication of pneumothorax, and of these only 37 per cent had mixed infections. A mixed infection makes the prognosis worse and usually arises from a bronchial fistula. Of the patients treated surgically, 51 per cent were "cured" and 23 per cent "improved". Of the 19 unoperated cases, one was "cured," one "improved" on penicillin, 3 were unimproved, and 14 died. In those cases in which operation was delayed longer than six months, there was a higher proportion of long-standing fistulae.—*Tuberculous empyema, D. A. Anderson, Brit. M. J., July 2, 1949, No. 4617: 6.*—(*R. W. Clarke*)

Tuberculous Empyema.—In a series of 63 cases of tuberculous empyema the results of treatment differed depending on the activity of the pulmonary disease. Of 17 patients with clinically inactive pulmonary lesions, 10 (59 per cent) recovered, 5 others (29 per cent) are still alive, and only 2 (12 per cent) died. Among 46 patients with active pulmonary lesions, only 9 (19.5 per cent) recovered, 12 others (26.5 per cent) are alive, 5 cannot be located, and 20 (43.5 per cent) died. Thoracoplasty without previous pleurotomy is the treatment of choice for unilateral lesions, closed empyemas without secondary infection, and inexpansible lungs. Drainage as the first step of the operation decreases the chances of success and should be reserved for cases of lung perforation. There were 51.5 per cent cures in 31 cases without pleurotomy, 9 per cent cures in 32 cases with pleurotomy.—*El tratamiento del empiema pleural tuberculoso, J. Teixeira, Rev. Pan. Med. & Cir. Tór., October–December, 1948, 1: 343.*—(*A. A. Moll*)

Thoracoplasty.—A new modification of thoracoplasty with apicolysis has been developed to obtain maximum collapse of the upper lobe and to prevent re-expansion. After resection of 5 ribs and apicolysis according to Semb's technique, 3 circular ligatures are applied around the upper part of the lung at different levels and attached to the intercostal muscles. The advantages of this procedure are that: (*1*) more collapse is obtained than with other methods; (*2*) fewer ribs are resected without impairing the collapse; (*3*) thoracic deformity is reduced. Eighteen cases have been operated with this method without complications.—*Thoracoplastie réduite avec apicolyse totale et ligature du sommet du poumon, F. Paulino, Presse méd., March 23, 1949, 57: 274.*—(*V. Leites*)

Thoracoplasty.—Results in 350 cases indicate that thoracoplasty, with or without apicolysis, in the treatment of fibrotic, ulcerated tuberculosis in the upper lung is preferable to pulmonary resection. With thoracoplasty there were 224 cures (64 per cent). Of 79 deaths, 6 were postoperative, 47 early, and 26 late. Tuberculosis was far advanced in 238 patients, moderately advanced in 110, and minimal in 2. Alexander's method of thoracoplasty and apicolytic thoracoplasty gave clinical cures in 69.3 and 63.15 per cent of cases, respectively. Alexander's method appeared to cause fewer immediate and late complications, but was followed more frequently by scoliosis. Sauerbruch's operation gave poor results, with only 42.85 per cent of clinical cures. It was mostly used, however, in very advanced bilateral cases.—*Ressecções polmonares e colapsoterapia cirúrgica no tratamento da tuberculose pulmonar, G. Martines-Botelho, Rev. brasil. de tuberc., March–April, 1949, 17: 119.*—(*A. A. Moll*)

Lung Function after Extrapleural Pneumothorax.—In 10 patients with pulmonary tuberculosis, functional examinations of the lungs were made before the induction of extrapleural pneumothorax and again at a period one and one-half to twenty months later. In 9 cases bronchospirometric values decreased on an

average as follows: complementary air, 39.9 per cent; reserve air, 38.8 per cent; vital capacity, 28 per cent; oxygen consumption, 24.3 per cent; respiratory volume, 7.6 per cent. Wide discrepancies prevailed between the different cases. The ventilation equivalent definitely decreased in most cases. In one of these cases a third bronchospirometry was performed several months after discontinuing and extrapleural pneumothorax which had been maintained for nearly three years. Partial functional recovery of the involved lung was shown. In the tenth case, an intrapleural pneumothorax was induced on the left side and an extrapleural one on the right. The former reduced functional capacity to a greater degree than the latter.—*Examen funcional de los pulmones por separado antes y después de instituído el neumotórax extrapleural, R. F. Vaccarezza, A. Soubrie, & J. C. Rey, Rev. Pan. Med. & Cir. Tór., January–March, 1948, 1: 141.*—(*A. A. Moll*)

Endoscopic Phrenicectomy.—In a sixteen-year-old girl with an artificial pneumothorax, an intrapleural pneumolysis with supplemental phrenic avulsion was advised. The patient objected for cosmetic reasons to the usual phrenic operation. The nerve then was approached endoscopically immediately after performing the pneumolysis. This approach can be tried only in cases of previous pneumothorax.—*Frenicectomia endoscópica, J. M. Castello-Branco, Clín. Tisiol., October–December, 1948, 3: 489.*—(*A. A. Moll*)

Pneumoperitoneum for Pulmonary Tuberculosis.—In 35 cases of pulmonary tuberculosis treated with induced pneumoperitoneum for a period ranging from four months to two years, the results generally were satisfactory. Of the group, 16 patients (45.7 per cent) were much improved with closing of all cavities, disappearance of tubercle bacilli from the sputum, and relief of symptoms; 13 (37.1 per cent) were improved with clinical and roentgenological regression but with cavities not quite closed and sputum still positive. In 25 cases, pneumoperitoneum was supplemented by crushing of the phrenic nerve. Streptomycin was used in 11 cases, 5 of them in the much improved group, 4 among the unimproved, and 2 of them failures. The location of the cavities did not influence the outcome. The only complication was the occurrence in 2 cases of small and subsequently absorbed effusions which did not interfere with the treatment.—*O pneumoperitônio no tratamento da tuberculose pulmonar, A. Ibiapina & A. Neves, Rev. brasil de tuberc., July–August, 1948, 16: 277.*—(*A. A. Moll*)

Pneumoperitoneum and Strangulated Hernia.—Artificial pneumoperitoneum may be associated with strangulated hernia By the use of trusses the pneumoperitoneum can be maintained satisfactorily in the presence of uncomplicated hernia.—*Artificial pneumoperitoneum associated with strangulated hernia and scrotal pneumocele, N. Wynn-Williams, Brit. M. J., August 6, 1949, 4622: 318.*—(*R. W. Clarke*)

Pneumothorax in Elderly Subjects.—Two patients aged 71 and 59 years, respectively, were given artificial pneumothorax for tuberculosis with good results. The limitations in elderly patients are chronic bronchitis and diminished cardiorespiratory reserve.—*Artificial pneumothorax in elderly subjects, E. H. W. Deane, Tubercle, August, 1949, 30: 179.*—(*A. G. Cohen*)

Osseous Tuberculosis of the Hand.—Tuberculous osteomyelitis of the short bones of the hand was observed in 15 male and 9 female patients. The highest incidence was in early youth with 39 per cent occurring during the first decade. Thus tuberculous involvement of the short bones may develop earlier than involvement of other parts of the skeletal system. The lateral half of the hand is usually affected and the phalanges of the middle fingers are most often involved. Involvement of more than one digit and its associated metacarpal is unusual. In the early stages the complaints are usually of discomfort rather than actual pain, followed by weakness and slightly in-

creased width of the short bones. Then a rather characteristic fusiform swelling develops. This is accompanied by mild atrophic changes in the overlying skin, most often in the dorsal aspect and may be followed by loss of motion of the digit. Musculature atrophy, "cold abscess" formation, and deep-seated pain may ensue. In mixed infection there is a more complicated picture with a tendency to involve the associated joint. The roentgenographic findings are those of productive or destructive changes in various combinations. Aspiration or incision and drainage of a "cold abscess" does not affect the underlying pathology and enucleation is required to cure the lesion in less than one and one-half years. Of 14 patients treated by aspiration or drainage, 6 died of accompanying generalized tuberculosis, 6 were improved or arrested after an average of 3 years, all of the latter being children. No adult improved on conservative therapy. The early surgical enucleation of each focus is indicated unless the procedure is considered too drastic. A longitudinal hemisection of the phalanx or metacarpal may arrest the lesion and avoid the deformity of multiple amputations when several such lesions occur in one hand.—*Tuberculosis of the short bones of the hand, M. Gershman, Quart. Bull., Sea View Hosp., October, 1948, 10: 146.*—(*K. T. Bird*)

New Types of Tomography.—Two new types of tomography have been developed and applied clinically. Simultaneous tomography results when the roentgen rays cross in the air, forming numerous sections with a single tube-film motion. These sectional pictures are transferred to a specially designed holder-press providing room for films, screen, and a set of cellophane divisions. Vibratory tomography, an extension of the above, is based on the principle that all sections located between two planes can be concentrated on the same film when projected simultaneously with vibration of about 1,000 per minute. Not a mere plane but a real "slice" develops, its thickness being contingent on the degree of vibratory motion. The method is said to provide a clearer view, increased contrast, elimination of dead spaces between individual tomographs, and sharp demarkation of the various structures in the picture. Obliquity of contrast surfaces to the roentgen beam impairs the value of the method.—*Tomografia vibratória, M. de Abreu, Clín. Tisiol., April–June, 1948, 3: 141.*—(*A. A. Moll*)

Clinical Studies, Nontuberculous Diseases

Chronic Beryllium Granulomatosis.—Ten cases of so-called chronic pulmonary granulomatosis associated with beryllium and its compounds occurred near a plant producing beryllium. Of these patients, 4 worked in the plant, 4 lived near the plant, one lived three miles from the plant where her husband was employed, and the other lived two to three miles from the plant and had only indirect contact with one employee. In 5 instances the disease manifested itself within eight months' postpartum. All cases had the typical insidious onset of mild productive or nonproductive cough followed by an acute coryza. The cough became more pronounced and dry, aggravated into paroxysms by sudden changes in air temperature and humidity. Exertional dyspnea was progressive, vital capacity diminished, chest expansion limited, and substernal pain produced at the height of inspiration. The symptomatic onset preceded the roentgenographic changes by several months in all cases. Each developed the characteristic ground-glass appearance in the chest roentgenogram with minute nodules throughout the lungs. No specific treatment is suggested. Two of the patients died. In 2 patients, 10 to 50 mg. of benadryl intravenously produced an amazing alleviation of acute symptoms immediately. The most effective treatment at present depends upon early limitation of exertion so that the patient's physical reserve is not impaired. Final convincing proof of the relationship of beryllium to chronic pulmonary granulomatosis is still lacking.—*Chronic pulmonary granulomatosis, J. M. DeNardi, H. S. Van Ordstrand, & M. G.*

Carmody, Am. J. Med., September, 1949, 7: 345.—(T. H. Noehren)

Value of Beryllium Determinations.—The clinical diagnosis of pulmonary berylliosis may be difficult and the detection of beryllium in the fluids or tissues might prove of value. Accordingly, the amount of beryllium in 24-hour samples of urine was determined spectrographically. Samples were used from patients who were ill with acute or chronic berylliosis, from healthy persons currently working in beryllium plants, and from patients who had recovered from acute pulmonary berylliosis. Some of the latter were again working in the industry. Results showed that persons who breathe in an atmosphere having beryllium-containing dust excrete detectable amounts of beryllium in the urine. The amount in the urine of apparently healthy persons exposed to the dust approximates the quantity present in the urine of those suffering from beryllium pneumonitis. The presence of beryllium in the urine, therefore, is an indication of the absorption of the metal, not of berylliosis. The content in the urine of persons with berylliosis varies from day to day so that a few negative analytical results cannot be accepted as excluding the disease. Appreciable quantities of beryllium were recovered from the lungs of all patients who died from acute or chronic pulmonary berylliosis, except for one person with the nonoccupational form of the disease. Also beryllium was found in renal calculi obtained from 2 patients with the disease.—*The value of beryllium determinations in the diagnosis of berylliosis, F. R. Dutra, J. Cholak, & D. M. Hubbard, Am. J. Clin. Path., March, 1949, 19: 229.—(J. S. Woolley)*

Nocardiasis.—A total of 37 cases of nocardiasis has been reported in the literature. Of these, 6 are known to have survived. The causative organism is an acid-fast sporothrix which goes under various names, the most recent of which is *Nocardia asteroides*. Two cases are reported in which good results were obtained with combined antibiotic therapy and surgical drainage despite the development of pneumonia and empyema. In material obtained directly from pus, the Ziehl-Neelsen stain shows short branching or single rods which are definitely acid-fast. In material taken from cultures, there are elements which are partially or irregularly acid-fast and also fine mycelial elements. After repeated cultures, the organisms tend to lose their acid-fastness. They are gram-positive; this property is lost by aging. The tissue lesions show no nodules or sulfur granules. The typical lesion is an abscess surrounded by an area of dense cellular fibrous tissue containing fibrin, neutrophils, lymphocytes, plasma cells, and macrophages with branching organisms scattered throughout. There are no caseation or giant cells. The disease usually begins in the lung and remains quiescent or asymptomatic for some time. Frequently it spreads to the pleura, causing an empyema with or without external sinuses. Frequently brain or subcutaneous abscesses appear. The most frequent symptoms are anorexia, weight loss, weakness, and a cough which becomes productive of greenish, foul, purulent sputum. Hemoptysis is rare. The condition, which may occur at any age, is readily confused with tuberculosis. Sulfadiazine or sulfamerizene and penicillin are effective especially when combined with surgical drainage.—*Nocardiasis, H. F. Hager, A. V. Gigliaccio, & R. M. Young, New England J. Med., August 11, 1949, 241: 226.—(A. G. Cohen)*

Pulmonary Arterio-sclerosis.—There are two forms of pulmonary arterial sclerosis, the form described from Brazil under the name of Ayerza's disease and pulmonary bilharziasis which has been extensively studied by the Faculty of Medicine in Cairo. In the latter type there is an obliterative endarteritis of the small branches of the pulmonary artery resulting from the action of ova which reach the site as emboli. With repeated reinfections the process extends centrally. Finally the main branches of the pulmonary artery may become dilated to aneurysmal proportions, and cor pulmonale develops. The lung parenchyma remains uninvolved. So long as the right ven-

tricle is able to force blood past the obstruction the lung can oxygenate it satisfactorily; cyanosis does not appear until the heart fails. There is, therefore, much less cyanosis than in the Brazilian type. In the latter instance most of the patients have been hard-rock miners at high altitudes and bilharziasis is unlikely to be the principal factor. *Bilharzia mansoni* does not have ova which reach the lung until after the development of bilharzial cirrhosis of the liver.—*Ayerza's disease, silicosis, and pulmonary bilharziasis, H. Tidy, Brit. M. J., June 4, 1949, No 4613: 977.—(R. W. Clarke)*

Pulmonary Fibrosis.—In an unusual case of fatal diffuse, progressive, interstitial fibrosis of the lungs, the clinical course of fifteen months consisted of two phases, latent and active, and was essentially afebrile throughout. There were no physical findings during the first period to explain the shortness of breath and cough. The roentgenogram, however, showed changes at the bases of both lungs suggesting pulmonary fibrosis. The active phase appeared suddenly with cyanosis, marked dyspnea, and increase of palpable activity over the right ventricle. Clubbing of the fingers appeared four weeks after the onset of cyanosis. At autopsy a great increase in the connective tissue was noted in the alveolar walls. A moderate degree of inflammation, recent and old, was found but there were no exudate or demonstrable organisms. The case is remarkably similar to that described in 1944 by Hamman and Rich.—*Diffuse progressive interstitial fibrosis of the lungs, A. J. Beams, & O. Harmos, Am. J. Med., September, 1949, 7: 425.—(T. H. Noehren)*

Pulmonary Embolism.—Post-mortem studies in 278 miscellaneous cases, excluding cases of Laënnec's cirrhosis, revealed 33 cases (12 per cent) of pulmonary emboli. No pulmonary emboli were found in 97 cases of Laënnec's cirrhosis, although cardiac and peripheral thromboses occurred as frequently in this as in the first group. Pulmonary embolism was found in 21 per cent of the patients over 70 years of age; it occurred in less than 7 per cent of the patients 50 to 70 years old, and did not parallel the incidence of thrombosis.—*Relation of pulmonary embolism to peripheral thrombosis, J. M. Spitzer, N. Rosenthal, M. Weiner, & S. Shapiro, Arch. Int. Med., September, 1949, 84: 440.—(G. C. Leiner)*

Intrapulmonary Vessels in Congenital Heart Disease.—The intrapulmonary vessels were studied at necropsy in 4 cases of coarctation of the aorta associated with patent ductus arteriosus. In 2 cases, the coarctation was distal to the aortic mouth of the ductus arteriosus; in 2 cases, the coarctation was proximal to the aortic mouth of the ductus arteriosus. In the first 2 cases, the intrapulmonary arteries showed medial hypertrophy, fragmentation of the elastic laminae, adventitial fibrosis, fibrous proliferation of the intima, and narrowing of the lumina. The arterioles showed thickened walls and narrow lumina. In the other 2 cases, the intrapulmonary arteries and arterioles showed medial hypertrophy, adventitial fibrosis, and narrowing of the lumina.—*Pathology of the intrapulmonary arteries and arterioles in coarctation of the aorta associated with patent ductus arteriosus, J. E. Edwards, J. M. Douglas, H. B. Burchell, & N. A. Christensen, Am. Heart J., August, 1949, 38: 205.—(G. C. Leiner)*

Cor Pulmonale.—A 55-year-old white man complained of episodic chest pain, shortness of breath, cough, and swelling of the ankles. The clinical diagnosis was right heart failure of undetermined etiology. The necropsy diagnoses were: (*1*) Anomaly of pulmonary artery with multiple aneurysms and band formations, (*2*) old massive thrombosis of both main branches of pulmonary artery, and (*3*) marked hypertrophy of right ventricle (chronic cor pulmonale).—*Contribution to the pathogenesis of chronic cor pulmonale: Report of a case with multiple aneurysms, intravascular bands, and old massive thrombosis of the pulmonary artery, G. E. Irvin, Am. Heart J., June, 1949, 37: 1144.—(G. C. Leiner)*

Acute Pulmonary Abscess.—The earlier the diagnosis of lung abscess is made, the better the chance for successful medical therapy. Three cases are reported where treatment was successful; 2 were putrid abscesses. Good postural drainage should be carried out four times a day. The patient should sleep without a pillow and the foot of the bed should be elevated. Penicillin 50,000 to 100,000 units should be injected every three hours; sulfadiazine should be administered in doses adequate to obtain a good level. Aerosol penicillin, 40,000 units dissolved in one cc. of water, should be given every four hours, preferably after postural drainage. If the organism is sensitive to streptomycin, this drug also should be given both parenterally and by aerosol. If satisfactory drainage is obtained and no regression occurs after four weeks, surgical intervention is indicated.—*Medical treatment of acute pulmonary abscess, W. J. Walker, New England J. Med., August 18, 1948, 241: 267.*—(*A. G. Cohen*)

Surgical Pulmonary Coccidioidomycosis.—Although the primary infection with *Coccidiodes immitis* usually is an acute benign respiratory infection subsiding in a few weeks, cavity formation occurs in about 6 per cent of the cases. The majority of the cavities close spontaneously in about six months and even if they remain patent the individual may lead a normal life. Pneumothorax has not given uniformly good results in attaining permanent closure. Surgery was required in 10 cases. Of 6 lobectomies performed, 4 were done because of persistent cough, low-grade fever, pleuritic pain, and hemoptyses associated with cavitation. One lobectomy was done because of an undiagnosed solid tumor and another because of an 8 cm. cavity with fluid level. Four decortications were performed because of nonexpansile lung following spontaneous pneumothorax. All surgery was performed without undue difficulty and there were no serious complications during or after the operations. There was no evidence of dissemination in any of the cases postoperatively.—*The surgical lesions of pulmonary coccidioidomycosis, S. J. Greer, & J. B. Grow, Dis. of Chest, September, 1949, 16: 337.*—(*E. A. Rouff*)

Pulmonary Adenomatosis.—Pulmonary adenomatosis is a rare irreversible condition consisting of hyperplasia and metaplasia of the alveolar epithelium into columnar glandular tissue. Histologically, it is benign but involves both lungs, eventuating in complete loss of respiratory function and death by asphyxia. Two cases are added to the previously reported 19 human cases. Some findings were common to all. (*1*) There was a long, slowly progressive, downhill course with pulmonary symptoms following an acute infection which never cleared completely. (*2*) There were exacerbations with partial remission of symptoms due to superimposed infections. (*3*) Roentgenograms showed a diffuse conglomerate pneumonic infiltration with little change over a period of months or years. (*4*) Sputum examinations revealed no causative organisms. (*5*) Grossly, the lungs were voluminous and heavy with raised, moist, gray patches of tumor. Microscopically, transformation of the normal alveolar lining by hyperplasia and metaplasia to a benign, mucus-producing columnar epithelium was seen. No clue as to the pathogenesis was found, although diminution of oxygen supply to areas of the lung is suspected.—*Pulmonary adenomatosis, S. Bubis, & J. H. Erwin, Am. J. Med., September, 1949, 7: 336.*—(*T. H. Noehren*)

Eosinophilic Xanthomatous Granuloma.—Although the nature of the Hand-Schüller-Christian syndrome is unknown, it has been thought to be a phase in a disease of the reticulo endothelial system and to have features in common with eosinophilic granuloma of bone. A 56-year-old man was first seen in 1942 because of progressive breathlessness on exertion; roentgenograms showed diffuse pulmonary shadows. In 1944, he fractured his femur through a cyst. In February, 1948, he began to have excessive thirst and polyuria, controlled by pituitary snuff, and laboratory investigation revealed true diabetes insipidus. Roentgenograms of the right femur showed a

cystic area beneath the old fracture; those of the chest revealed generalized reticulation with miliary mottling throughout both lungs. The skull showed irregular translucencies resembling exaggerated venous channels rather than lipoid deposits. A biopsy specimen of the femur disclosed a typical eosinophilic granuloma of the bone. The characteristic radiographic changes of honeycomb lungs shows this to be an example of cystic pulmonary changes occurring in the more chronic phase of esinophilic xanthomatous granuloma. That pulmonary infiltrations occur in this disorder, has been recognized since 1929.—*Eosinophilic zanthomatous granuloma with honeycomb lungs, T. Parkinson, Brit. M. J., June 11, 1949, No. 4614: 1024.—(R. W. Clarke)*

Pleural Mesothelioma.—A case of combined pleural and peritoneal mesothelioma was a diagnostic enigma for four and one-half years. The presenting symptoms were a chronic productive cough, vague abdominal pain, and gradual weight loss. Signs of fluid in the left chest, an enlarged supraclavicular node, and an enlarged spleen were the only abnormal physical findings. The thoracentesis fluid was blood-tinged; other laboratory studies were negative. Sputum examination, thoracoscopic biopsy of the pleura, abdominal exploration with lymph node biopsy, paracentesis with cytologic studies, and therapeutic trials of roentgen and mustard therapy all failed to reveal the diagnosis. At autopsy a mesothelioma of the pleural surfaces on the left was found to have obliterated the pleural space; involved the peritoneum with extension into the pancreas, posterior wall of the stomach and transverse colon; and extended to the left tracheobronchial, bronchopulmonary, and mesenteric lymph nodes. Histologically mesothelioma is characterized by the absence of mucin within the cells, as contrasted with carcinoma originating from mucin-secreting organs like the bronchus.—*Chronic pleurisy and peritonitis, Clinico-pathologic Conference, Am. J. Med., September, 1949, 7: 396.—(T. H. Noehren)*

Intrathoracic Neurogenic Tumors.—During the past 23 years, 9 cases of intrathoracic neurogenic tumors were operated upon. In 8 cases, there had been symptoms of pressure in the chest, pain, dyspnea, and cough; in one case, there had been no symptoms. The surgical removal of the tumor is indicated, even in asymptomatic cases, because of the danger of malignant degeneration. The transthoracic intrapleural approach is the method of choice. Three patients died subsequent to the operation from exsanguination. Six patients are well 9 months to 23 years after the operation. The largest tumor measured 14 by 13 by 8 cm.; its weight was 825 Gm.—*Intrathorakale neurogene Tumoren, H. Homma, Wien. klin. Wchnschr., July 8, 1949, 61: 421.—(G. C. Leiner)*

Needle Aspiration in Lung Cancer.—During the past eight years needle aspiration of tissue from lung lesions has been used as a diagnostic procedure 75 times on 56 of 145 patients suspected of having lung carcinomas. The indications were: (*1*) failure or inadvisability of bronchoscopy and other techniques, (*2*) obvious inoperability where curate diagnosis was of great importance, and (*3*) peripheral location of tumors. An 18-gauge 10 cm. spinal needle with stylet was used. By this method a positive diagnosis was obtained in 44 of the 56 patients (78.5 per cent). In 3, a mild degree of pneumothorax resulted with no untoward results; 5 patients had transient blood spitting.—*Needle aspiration in the diagnosis of lung carcinoma, report of experience with seventy-five aspirations, E. Y. Gledhill, J. B. Sprigg, & C. H. Binford, Am. J. Clin. Path., March, 1949, 19: 235.—(J. S. Woolley)*

Treatment of Postoperative Atelectasis.—As soon as postoperative atelectasis is diagnosed, the position of the patient's bed is changed so as to promote drainage. The patient is encouraged to try to clear the chest. Procaine is slowly injected intravenously until there is numbness, tingling, or twitching; then the patient is told to cough. Simultaneously, the chest is percussed violently. Twenty cc.

of 0.5 per cent procaine are used initially, about one-half being injected in two to three minutes. The needle is kept in the vein and a little more is given if no effect is obtained. No barbiturates are given initially but a syringe full of a suitable preparation is kept available in case convulsions should appear. The important point is to use the procaine early. The authors have used it in many cases with good results.—*Intravenous procaine for postoperative atelectasis, A. H. M. Siddons, & E. Landau, Lancet, September 3, 1949, 2: 419.*—(*A. G. Cohen*)

Spondylarthritis.—In ankylosing spondylarthritis the widespread impairment of joint function includes the costovertebral joints. The resulting fixation of the thoracic wall leads to disturbance of respiratory function and marked impairment of the cough reflex. These in turn cause retention of bronchial secretions and chronic recurrent pulmonary infection frequently associated with areas of atelectasis. In cases of chronic pulmonary infection not adequately explained, the possibility of ankylosing spondylarthritis must be kept in mind. Simple upper respiratory infections frequently lead to serious results in this group of patients and should be carefully treated. When abdominal operations are necessary, great care is indicated to prevent serious respiratory complications. In using plaster casts to treat orthopedic deformities, every attempt should be made to prevent further immobilization of the chest. Training and exercises in thoracic breathing are important preventive measures.—*Pulmonary disease manifestations of ankylosing spondylarthritis, K A. Hamilton, Ann. Int. Med., August, 1949, 31: 216.*—(*H. R. Nayer*)

Fracture of Bronchus.—A patient sustained a nonpenetrating injury of the chest. After a few days, she developed dyspnea and wheezing and there was evidence of bronchial obstruction. Bronchoscopy showed a stricture of the left main bronchus which was interpreted as secondary to a fracture of the bronchus. Attempts at dilation were futile. The patient was operated upon and a segment of the bronchus was resected. The cut edges were then joined by end-to-end anastamosis. The patient made a good recovery.—*Fracture of the bronchus, J. L. Griffith, Thorax, June, 1949, 4: 105.*—(*A. G. Cohen*)

Antimicrobial Therapy

Crystalline Streptomycin.—Crystalline streptomycin alone was used in 44 patients with advanced pulmonary and extrapulmonary tuberculosis for six months and the results compared to a control group who had received streptomycin hydrochloride, sulfate, or calcium chloride in the previous year. Another 11 patients received crystalline streptomycin after having had treatment with one of the other forms of streptomycin. In these few cases, no differences could be detected between the immediate therapeutic effects of crystalline streptomycin and of the noncrystalline form. The toxic effects were of about the same general severity and character; the crystalline form was less painful locally and was less often associated with febrile reactions. The finding of bacterial resistance to streptomycin is not felt to be of paramount importance clinically since many patients whose organisms exhibit high degrees of resistance show definite therapeutic improvement. In a chronic disease like tuberculosis, the resistance of the patient is more decisive in determining the eventual outcome than drug resistance acquired by the tubercle bacilli. *Streptomycin in tuberculosis, E. H. Rubin, M. M. Steinbach, G. C. Leiner, L. Adelsberger, & H. M. Zimmerman, Dis. of Chest, September, 1949, 16: 304.*—(*E. A. Rouff*)

Streptomycin and Pneumothorax.—Within the past two years, 12 patients with cavitary, pneumonic, and bronchopneumonic tuberculous lesions, accompanied by marked constitutional involvement, were treated at the earliest possible moment with streptomycin and pneumothorax. During a preparatory period of ten to fourteen days, 2 Gm. of streptomycin and one to two million units of penicillin were given daily. This produced defervescence, a notable decrease in the density of the infiltrations, diminution in the

size of cavities, and an improvement in the general condition. Pneumothorax was instituted after two weeks, streptomycin being continued until a total dose of 200 to 400 Gm. had been given. This first or "urban" phase was followed by a phase of treatment in a country sanatorium with continuation of pneumothorax treatment. The results obtained are considered so significant from the therapeutic, prophylactic, and public health point of view that it would be regrettable to deprive patients of such treatment for fear of producing streptomycin resistance.—*Streptomycine et pneumothorax associés dans le traitement de la tuberculose pulmonaire fibro-caséeuse de l'adulte, Ch. Mattei, M. Tristani, P. Balozet, & C. Mattei, Presse méd., June 15, 1949: 537.*—(*V. Leites*)

Streptomycin for Childhood Tuberculosis.—Eight children with tuberculous bronchopneumonia were treated with streptomycin; 2 died, 6 survived and have been followed for a period of six to twenty-two months. The children were ill for 27 to 90 days before admission. All were obviously short of breath, 7 had coughed for several weeks. There was pallor in 7, bronchitis in 4, consolidation in 3. Roentgenograms showed patchy shadowing of the lung fields in all 8. All had tubercle bacilli in the sputum or stomach washings. All except one had a positive tuberculin reaction, and he was negative to a weak dose only. The patients were given streptomycin, 0.02 Gm. per pound, and a reaction was observed in only one patient. In 3 of the cases, tubercle bacilli were found after the beginning of treatment and there was no increase in resistance to streptomycin. In one child, tuberculous meningitis developed during treatment; the organisms became resistant.—*Streptomycin treatment of tuberculous bronchopneumonia in childhood, R. McL. Todd, Brit. M. J., April 30, 1949, No. 4608: 741.*—(*R. W. Clarke*)

Streptomycin for Tuberculous Meningitis.—The interim report of the Streptomycin Subcommittee of the Scottish Scientific Advisory Committee describes 81 cases of tuberculous meningitis and their treatment between September, 1947 and May, 1948. Tubercle bacilli were isolated from the spinal fluid in 77 cases. No case responded to intramuscular streptomycin alone. Of the 81 patients, 45 are dead, 5 have relapsed or are deteriorating, and 31 are progressing favorably.—*Tuberculous meningitis treated with streptomycin (Editorial), Brit. M. J., April 9, 1949, No. 4605: 628.*—(*R. W. Clarke*)

Streptomycin and Hydrocephalus.—The leptomeninges from the undersurface of the pons were available for histological examination from 8 cases of tuberculous meningitis; 6 of these with hydrocephalus and 2 without. Intramuscular and intrathecal streptomycin had been given 7 of the patients for periods of 12 to 231 days. One hydrocephalic patient received no antibiotic therapy. The basal subarachnoid space in all of the hydrocephalic cases was completely blocked by a fibrinous exudate and thick tuberculous granulation tissue. Two of the 5 treated hydrocephalic cases showed some fibroblastic exudate. The other 3 gave no evidence of organization nor was there any obvious histological modification of the lesions by streptomycin when compared with the lesions from untreated cases. The hydrocephalus was of the communicating type, the subarachnoid space beneath the pons being filled with fibrinous and caseous exudate and tuberculous granulation tissue. In the 2 treated nonhydrocephalic cases, the subarachnoid channels were patent. It is felt that tuberculous meningitis alone can give rise to hydrocephalus; the prominence of the condition since the introduction of streptomycin treatment is due to more careful examination of the brain. There is no evidence that streptomycin increases the chance of hydrocephalus by stimulating the production of reparative tissue.—*Streptomycin in development of hydrocephalus in tuberculous meningitis, S. N. De, Brit. M. J., July 23, 1949, No. 4620: 214.*—(*R. W. Clarke*)

Streptomycin Toxicity.—Damage to the auditory and vestibular apparatus can be

avoided if streptomycin dosage is related to body weight. In the adult it is inadvisable to administer more than 24 mg. per Kg. of body weight. If 20 mg. per Kg. is given intramuscularly daily, damage to the auditory apparatus is unlikely and any vestibular disturbances usually are transitory. With a daily dose of 16 mg. per Kg. or less, damage to the vestibular apparatus is not apt to occur. Children can tolerate large doses much more readily without toxic manifestations. Impaired kidney function may produce serious toxic symptoms even without high dosage. When hearing is affected, the higher frequencies are lost first but, as the damage becomes more serious, all frequencies are affected. While impaired vestibular function often improves partially or completely upon cessation of therapy, this is not true of hearing. More involvement of the eighth nerve is seen in meningitis than in pulmonary tuberculosis; it is not clear whether this is due to the illness or the intrathecal streptomycin therapy. Experimentally, certainly eighth nerve damage of high degree can be produced by the administration of intramuscular streptomycin alone.—*Streptomycindosierung und neuretoxische Nebenwirkungen, K. Graf., Schweiz. med. Wchschr., September 3, 1949, 79: 793.*—(*M. Marcus*)

Visual Disturbances with Streptomycin.—Among 52 children with tuberculous meningitis treated with streptomycin, visual disturbances were observed in 8. In 5 of these, they were due to atrophy of the optic nerve. In 4 children with visual disturbances, of which 3 had atrophy of the optic nerve, arachnoiditis optochiasmatica was diagnosed on clinical or pathological grounds. In 2 cases sudden temporary blindness occurred. This was caused by an acute hydrocephalus. Acute hydrocephalus is seen occasionally in cases of tuberculous meningitis treated with streptomycin. *Ueber Sehstörungen bei der Streptomycinbehandlung der Meningitis tuberculosa, F. Mejer, Wien. klin. Wchnschr., July 1, 1949, 61: 407.*—(*G. C. Leiner*)

Thiosemicarbazones for Tuberculosis.—Numerous derivatives of thiosemicarbazones were tested *in vitro* and in *vivo* for antituberculous activity. None of the mice infected with tuberculosis by the intravenous route were completely protected by the treatment. Significant extension of survival time for treated animals as compared to untreated controls was found with certain compounds of the class of substituted benzaldehydes and heterocyclical aldehydes of thiosemicarbazone. Five compounds were found to be more effective than para-aminosalicylic acid. The general structural formula of this class of compound is as follows: R-phenyl-CH:N·NH·CS·NH_2. The most effective groups at the R position were: CH_3O, OH, $C_2H_5SO_2$. Toxicity was low.—*Studies in the Chemotherapy of tuberculosis: Part V. Thiosemicarbazones and related compounds, E. Hoggarth, A. R. Martin, N. E. Storey, & E. H. P. Young, Brit. J. Pharmacol., September, 1949, 4: 248.*—(*A. A. Cohen*).

Blood Concentration of Thiosemicarbazones.—Spectrophotometric methods of measuring blood levels of 3 antituberculous thiosemicarbazones have been devised. The *p*-ethylsulfonylphenyl compound (R = $C_2H_5SO_2$) produced higher and more persistent blood concentrations than the *p*-hydroxyphenyl (R = OH) or *p*-anisyl (R = CH_3O) compounds. Significantly, the *p*-ethylsulfonylphenyl compound is more effective *in vivo* than the other two.—*The estimation of some thiosemicarbazones and their blood concentrations in experimental animals, A. Spinks, Brit. J. Pharmacol., September, 1949, 4: 254.*—(*A. A. Cohen*).

Surface-active Agents via Aerosol.—Surface-active agents lower surface or interfacial tension because one portion of the molecule is hydrophilic; another, lipophilic. The lipophilic portion of the surface-active molecule may attach to the lipids at the surface of the bacterial cell while the hydrophilic portion allows greater penetration of the cell by water-soluble substances. Streptomycin is soluble

in water but not in oil. As tuberculous caseous exudate contains much lipid material, a surface-active agent may also emulsify this material. Streptomycin is relatively ineffective in the presence of tuberculous caseation and of tissues, like the brain, which are high in lipid content. Trition A-20 (alkyl aryl polyether alcohol) and Tween-20 (polyoxyethylene sorbitan monolaurate) were found to be nontoxic to mice in acute and chronic feeding tests, and then were used in the streptomycin solution administered as an aerosol to 2 patients with pulmonary tuberculosis. In all, 3 humans were given daily aerosol treatment with these surface-active agents for periods up to 95 days without observable ill effects. It is hoped to determine whether the high and prolonged levels of streptomycin in the lungs obtainable by aerosol administration, together with the action of the surface-active agents on caseous material and tubercle bacilli, will be of value in decreasing the present limitations of systemic streptomycin therapy of pulmonary tuberculosis.—*The applicability of surface-active agents in aerosol streptomycin therapy of pulmonary tuberculosis, J. B. Miller, Quart. Bull., Sea View Hosp., October, 1948, 10: 155.*—(*K. T. Bird*)

Manganese Chloride for Tuberculous Meningitis.—A 4-year-old girl and a 26-year-old man with tuberculous meningitis were treated by rectal administration of 1.0 cc. of 0.02 molar solution of manganese chloride once or twice daily. Both patients were discharged as cured. Thirteen years later the first patient is healthy, the second has fibrocavernous tuberculosis of the lungs.—*Hämatogene Tuberkulose und Manganchlorid, V. Gorlitzer von Mundy, Wien. klin. Wchnschr., August 26, 1949, 61: 539.*—(*G. C. Leiner*)

Ephedrin-Penicillin Inhalation.—Comparative therapeutic studies were made on each of two series of 14 patients with chronic bronchial infection, chiefly with bronchiectasis, emphysema, and intrinsic asthma. One series was treated for three weeks with nebulized ephedrine sulfate U.S.P. followed by aerosol of penicillin calcium. The patients gained considerable benefit, indicated by reduced volume of sputum, increased exercise tolerance, reduced dyspnea, cough, and chest discomfort, improved sleep and appetite, and decreased epinephrine requirement. The other series was treated for an equal period with micronized ephedrine sulfate U.S.P. followed by micronized penicillin calcium. These patients by the same criteria apparently were equally benefited and in addition had increased vital capacity. Minor reactions such as oral dryness, saccharine taste, and nausea appeared in this group. Allergic manifestations rarely accompanied the therapy. Inhalation of micronized ephedrine will frequently replace the hypodermic administration of epinephrine for asthma. Bacteriologic studies indicated approximately equal effectiveness of moist and dry therapy.—*Aerosol and micronized ephedrine and penicillin therapy of diseases of the lower respiratory tract. Treatment of bronchitis, bronchiectasis and intrinsic asthma, S. J. Weinberg & G. L. Packer, Arch. Int. Med., September, 1949, 84: 389.*—(*G. C. Leiner*)

Laboratory Studies

Tumor Cells in Bronchial Secretions.—An experimental study on rabbits was made to determine (*1*) how soon after the establishment of tumor growth tumor cells could be identified in bronchial secretions, and (*2*) what proportion of experimental animals yielded positive smears. The Brown-Pearce carcinoma, a readily transplantable tumor of the testis of the rabbit, was established in the bronchi of 30 rabbits and the bronchial secretions, obtained by tracheal swabs, were studied by the Papanicolaou technique. Smears were taken every third day, beginning on the sixth day following transplantation. In 17 of the animals, or 58 per cent, positive smears were found on the sixth day. By the end of the third week, all the animals showed positive smears; in some instances as many as 10 to 12 smears had to be taken before tumor cells could be identified in the bronchial secretions. In order to correlate the appearance of tumor cells in the bronchial secretions with the

appearance of grossly recognizable tumor in the lung, the animals were sacrificed as soon as positive bronchial smears were obtained. In none of the 17 animals showing exfoliated tumor cells at six days could tumor be recognized grossly. At this stage small microscopic nodules of tumor could be seen but no gross evidence of tumor was found. The earliest time at which tumor could be recognized grossly was 10 or 12 days following transplantation. Thus in 58 per cent of the rabbits exfoliated tumor cells were found before the tumor reached a stage at which roentgenograms or bronchoscopy could have identified the growth.—*Tumor cells in bronchial secretions: An experimental and statistical study, M. Appel & T. T. Bronk, Am. J. Clin. Path., April 1949, 19: 320.—(J. S. Woolley)*

Cytohistology of Bronchial Secretions.—Much progress has been made recently in the early diagnosis of bronchial carcinoma by the study of secretional smears. Yet there is little to be found in the American literature concerning the embedding and sectioning of material aspirated through the bronchoscope. Two series of patients are presented in whom a diagnosis of pulmonary neoplasm was considered. Wherever bronchoscopy was done, aspirated material was sent to the laboratory for section. All the material collected in the bronchoscopic trap was immediately fixed in formalin. At the laboratory the specimen was placed in a solution of saturated picric acid for four hours. It was next poured onto folded filter paper, the solidified material packed into the cone, treated like any other tissue, and embedded in paraffin. In one series carcinoma cells were found in 10 (66 per cent) of 15 specimens whereas only 5 (55 per cent) of 9 sputum smears were positive. In the other series 17 (89 per cent) of 19 bronchial aspirations yielded positive findings by the embedding technique. Apparently the smear method was not regularly done in this group. The authors believe that the cytohistological study of bronchial secretions is equal, if not superior, to the smear technique if all the material obtained is utilized and fixed at once.—*A cytohistological study of bronchial secretions, H. L. Richardson, W. C. Hunter, W. S. Conklin, & A. B. Petersen, Am. J. Clin. Path., April, 1949, 19: 323.—(J. S. Woolley)*

Alveolar Air.—By means of an equation the alveolar gas concentration can be expressed in terms of the relative alveolar ventilation and pulmonary blood flow. A graphic representation indicates all the concentrations of oxygen and carbon dioxide which could exist for any given tensions of these gases in venous blood and inspired air. Each possible value for alveolar air and, therefore, each respiratory quotient is determined by the ratio of ventilation to blood flow. If the respiratory quotient is known, the theoretical composition of the mean alveolar air can be defined by the equation. This theoretical concept of mean alveolar air presupposes that the ratio of ventilation to blood flow is the same in all the alveoli. Since this is unlikely, a variation of the ratio along a normal distribution curve is assumed. The value for such a mixture of mean alveolar air does not differ appreciably from the theoretical mean. The resulting mixture of arterial blood, however, yields a lower pO_2 tension, thus giving rise to an alveolar-arterial oxygen gradient. Evidence is presented which indicates that the sampling of alveolar air from the last part of each normal expiration yields a value which is very close to the theoretical composition of mean alveolar air, while the Haldane method of sampling yields carbon dioxide values which are slightly too high and respiratory quotients which are slightly too low. The latter method is responsible for the difference in the volume of the dead space when calculated separately from the oxygen and carbon dioxide values. Theoretically the volumes should be the same.—*A concept of mean alveolar air and the ventilation: Blood flow relationships during pulmonary gas exchange, H. Rahn, Am. J. Physiol., July 1, 1949, 158: 21.—(G. C. Leiner)*

Carbon Dioxide Inhalation in Emphysema.—Quantitative studies on the response of the

ventilation to carbon dioxide inhalation were carried out on 12 normal subjects, 12 patients with emphysema, and one patient with fibrosis of the lung (Boeck's sarcoid). Tidal air, respiratory rate, minute ventilation, arterial serum carbon dioxide, and pH determinations were made before and during hyperpnea produced by addition of carbon dioxide to the spirometer. Minute liter ventilation increase, divided by the millimeter increase in tension of carbon dioxide of the normal group, showed an average increase of 2.71 liters per minute for each millimeter of mercury increase of carbon dioxide, or a median value of 1.6 liters. In terms of pH this was shown to be an increase of 3.16 liters per minute for each 0.01 decrease in pH, or a median value of 2.5 liters. The patients with emphysema were shown to have an average of 3.0 volumes per cent (7.0 per cent) more total carbon dioxide content than did the normal subjects. The average response was 1.85 liters per minute increase in ventilation for each millimeter increase in carbon dioxide tension (66 per cent that of the normal group) when both groups were considered as a whole. There was a wide variation in the response within each group, and considerable overlapping between the response of the 2 groups existed. A statistical analysis would seem to indicate that the difference was not significant. However, the patients with emphysema did not get as much tidal exchange for a given amount of respiratory effort as did the normal subjects, and were shown by the relationship $\frac{\text{ventilation (in liters per minute)}}{\text{vital capacity (in liters)}}$ to have an increased resting respiratory effort as well as a greater increment of respiratory effort for a given change in pH consequent to carbon dioxide inhalation. From the evidence presented it seems apparent that in terms of respiratory effort the emphysematous patient is actually more sensitive to carbon dioxide inhalation than the normal individual. The explanation for this phenomenon is unknown at the present time (Authors' summary).—*Quantitative studies of ventilation during inhalation of carbon dioxide in normal and emphysematous patients, J. Holman & G. T. Shires. Am. Heart J., June, 1949, 37: 1101.—(G. C. Leiner)*

Respiratory Acclimatization to Altitude.—With the aid of an oxygen-carbon dioxide diagram, data are presented allowing one to predict the alveolar oxygen-carbon dioxide composition as well as the relative alveolar ventilation (*1*) when man is exposed acutely to any altitude, (*2*) during the process of respiratory acclimatization at any altitude, and (*3*) when acclimatization is complete. In addition, the alveolar pathways can be predicted and described once man is acclimatized to any particular altitude and is then suddenly exposed to higher or lower oxygen pressures. These predictions were in part verified by an acclimatization study carried on at an altitude of 9,500 ft. for a three-week period. Evidence is presented for a pO_2 threshhold of the chemoreceptor drive of ventilation at approximately 100 mm. of mercury. This is observed in the ventilation curve of people acclimatized to altitude. However, in acute exposures where the alveolar pO_2 is reduced to 50 to 60 mm. mercury this hyperventilation response is completely inhibited for at least one hour. This inhibition is explained as a result of the pH rise due to the decreased oxyhemoglobin saturation. If the alveolar pO_2 in acute exposure falls below 50 to 60 mm. of mercury, immediate hyperventilation occurs. Exposures of more than one hour at 9,500 ft. result in a lowering of the pCO_2 exponentially with time. Final levels are reached after three or four days. After respiratory acclimatization to 9,500 ft. the respiratory system becomes more sensitive to carbon dioxide. This response was tested by breathing various carbon dioxide mixtures and analyzing the alveolar air after breath holding. Data are presented which show the reduction of breath-holding time during acclimatization to various altitudes. The various findings emphasize that a large part of the respiratory acclimatization to high altitudes is an adaptation to a lowered carbon dioxide tension. (Authors' summary.)—*Man's respiratory response during and after acclima-*

tization to high altitude, H. Rahn & A. B. Otis, Am. J. Physiol., June 1, 1949, 157: 445.—(*G. C. Leiner*)

Respiratory Water Vapor.—The vapor pressures of exhaled gas have been calculated from gravimetric data and from dew-point measurements. Fifteen subjects at rest breathing oxygen have been tested at ground level and at a pressure altitude of 30,000 feet. At that altitude the decrease in gas volumes corrected to exhaled conditions was approximately the same as the decrease in the rate of water loss, so that the computed vapor pressures did not significantly change from ground level to high altitude. The concomitantly measured dew-points of exhaled gas showed at high altitude a statistically significant decrease in vapor pressure of almost 2 mm. of mercury. When corrected to standard conditions (0° C., dry, ambient pressure), the respired volumes decreased proportionately more than did the water output, so that the calculated mean vapor pressure at high altitude was approximately 3 mm. of mercury higher than at ground level. Correlation coefficients suggest that respiratory rate changes are more important in determining the aqueous vapor pressures than are tidal and minute volumes. (Authors' summary).—*Respiratory water vapor at simulated altitude, L. A. Marshall & H. Specht, Am. J. Physiol., March, 1949, 156: 299.*—(*G. C. Leiner*)

Vaccine Effects on Respiration.—Respiratory rate and minute volume were determined in rabbits injected with typhoid-paratyphoid vaccine. Administration of the vaccine did not significantly alter the degree of respiratory stimulation by the inhalation of 5 per cent carbon dioxide. It increased the respiratory minute volume in anesthetized animals and probably also in unanesthetized animals. In the latter this effect may be masked by suppression of thermal polypnea.—*Typhoid-paratyphoid vaccine and respiratory response to the inhalation of carbon dioxide, F. A. Ellis, R. Grant, & V. E. Hall, Am. J. Physiol., July 1, 1949, 158: 16.*—(*G. C. Leiner*)

Lung Changes in Hyperthermia.—In anesthetized guinea pigs under artificial respiration, hyperthermia caused by a warming box favored the production of pulmonary edema, hemorrhage, and congestion. When the animal breathed completely humidified air warmed to body temperature, the results were not altered.—*Effects of changes in body temperature and inspired air humidity on lung edema and hemorrhage, F. J. Haddy, G. S. Campbell, & M. B. Visscher, Am J. Physiol., September 1, 1949, 158: 429.*—(*G. C. Leiner*)

Ammonium Pulmonary Edema.—Pulmonary edema was produced in rats and guinea pigs by administration of a 6 per cent ammonium chloride solution either by gavage or by intraperitoneal injection. Central nervous system depressants, such as pentobarbital, evipal, morphine, chloralose, chloralosane, or ether did not prevent the occurrence of ammonium pulmonary edema nor did ligation of the common carotid arteries. Bilateral cervical vagotomy, atropinization, or adrenalectomy did not prevent ammonium edema; dibenamine had some protective effect. Decortication did not prevent edema in most animals but mesencephalic decerebration had a protective effects. Spinal cord section above the second thoracic segment protected the animals; sections between the third and seventh thoracic segments had some protective effect; sections below the twelfth thoracic segment had no protective effect. Systemic hypertension occurred following ammonium administration but was not a primary factor in the genesis of pulmonary edema. The alarm reaction, induced by exposure to cold and by subcutaneous injection of formalin, protected most rats from ammonium pulmonary edema. —*Studies on the pathogenesis of ammonium pulmonary edema, H. Koenig & R. Koenig, Am. J. Physiol., July 1, 1949, 158: 1.*—(*G. C. Leiner*)

Diphosgene Action on Lung.—The effect of diphosgene, which is similar to that of phosgene, was studied in dogs. Diphosgene produces demonstrable pathological changes in

the lung only after direct contact. No toxic agent is released. Bronchial narrowing with partial functional obstruction leading to pulmonary emphysema is seen after gassing. There is no definite evidence that diphosgene damages the alveoli. Death after moderate doses of diphosgene is due to anoxia caused by pulmonary edema; death after massive exposure is due to occlusion of the pulmonary circulation from clotting and hemolysis.—*Localization of the site of action of a pulmonary irritant, diphosgene, J. M. Tobias, S. Postel, H. M. Patt, C. C. Lushbaugh, M. N. Swift, & R. W. Gerard, Am. J. Physiol., August 1, 1949, 158: 173.*—*(G. C. Leiner)*

Respiration and Circulation.—Simultaneous registration of respiration and intra-arterial pressure revealed an unknown relation between respiration and blood pressure. Changes in inspiratory and expiratory position of the chest affect the blood pressure. During a deep inspiration the systolic pressures decrease more than during regular inspirations. With an increased expiratory position of the chest the systolic and diastolic blood pressures decrease. With the gradual return of the chest to its midposition the blood pressures gradually return to the previous levels.—*Ueber einen neuen Regulationsmechanismus zur Koordination von Atmung und Kreislauf, H. Schröcksnadel, Wien. klin. Wchnschr., April 8, 1949, 61: 214.*—*(G. C. Leiner)*

Respiratory Pathways.—In acute experiments on decerebrated or anesthetized cats and rabbits, a spinal lesion approaching or exceeding a hemisection was made at the second cervical level. The discharge of the phrenic motoneurons on each side of the cord was then measured by simultaneous action potential records from each hemidiaphragm. Controls established the absence of significant interaction between the two recordings. In each of 8 cats, significant crossed (i.e., ipsilateral to lesion) diaphragmatic activity occurred in the control state even when the spinal lesion far exceeded a hemisection. This crossed activity could be completely and differentially abolished by procedures which decrease respiratory effort, e.g., hyperventilation, and are augmented by procedures which enhance respiratory effort e.g., rebreathing, negative intratracheal pressure. Similar results were obtained for those 4 of the 8 rabbits which exhibited crossed respiration in the control state. Procedures which enhance respiratory effort elicited significant crossed activity in the remaining rabbits. In conclusion, bulbospinal fibers of respiratory function descend on the intact side of the cord and make connections with phrenic motoneurons of the opposite side. These connections are quantitatively less powerful than the uncrossed ones. Intensity of discharge from the respiratory center is an important factor determining whether impulses in the crossing pathways alone will effectively excite the phrenic motoneurons. (Authors' summary.)—*Descending respiratory pathways in the cervical spinal cord, H. Rosenbaum & B. Renshaw, Am. J. Physiol., June 1, 1949, 157: 468.*—*(G. C. Leiner)*

Pulmonary Effects of Intracranial Pressure.—Elevation of the intracranial pressure in anesthetized dogs was found to be followed ordinarily by: bradycardia, lowered cardiac output, and increased mean pulmonary venous and arterial pressures. These effects were independent of whether there was spontaneous or artificial respiration. When the pulmonary venous pressure rose above 20 mm. of mercury, significant degrees of pulmonary edema, congestion, and hemorrhage were seen at necropsy. In some instances such lesions were seen at lower venous pressures. Administration of atropine after induction of the changes listed above as following maintained elevation of intracranial pressure reversed those changes, and significant pulmonary edema did not occur. (*Authors' summary.*)—*Circulatory changes and pulmonary lesions in dogs following increased intracranial pressure, and the effect of atropine upon such changes, G. S. Campbell, F. J. Haddy, W. L. Adams, & M. B. Visscher, Am. J. Physiol., July 1, 1949, 158: 96.*—*(G. C. Leiner)*

Pulmonary Vascular Pressures.—In anesthetized dogs right heart catheterizations were done through the jugular vein; the pulmonary veins were catheterized through the carotid artery. There were 7 deaths in 71 trials of left heart catheterization. There were significant positive correlations between systemic arterial pressure and (*1*) mean pulmonary arterial pressure, (*2*) pulmonary venous pressure, and (*3*) cardiac output; between pulmonary venous pressure and cardiac output. There was no significant correlation between systemic venous pressure and cardiac output.—*A Study of pulmonary venous and arterial pressures and other variables in the anesthetized dog by flexible catheter techniques, F. J. Haddy, G. S. Campbell, W. L. Adams, & M. B. Visscher, Am. J. Physiol., July 1, 1949, 158: 89.—(G. C. Leiner)*

Concentration of Acid-fast Bacilli.—Of the many agents used to prepare sputum concentrates, sodium hypochlorite seems the most desirable. The commercial salt is subject to deterioration, but a simply prepared mixture was stable when refrigerated for three to six months. Prolonged stirring of digested sputum was of no advantage whether it was to be examined by direct smear or by concentration. When graded amounts of a fine suspension of tubercle bacilli were added to a negative sputum and then examined, concentration with sodium hypochlorite resulted in a tenfold increase over the direct smear method in the number of bacilli found. The wetting agent, Tergitol 08, alone or in combination with sodium hypochlorite, exerted a clear or gelatinizing action upon tuberculous sputum. This differed from the effect of sodium hypochlorite which acted as a digestant and resulted in a sediment of low volume with an opalescent supernatant fluid. The addition of Tergitol 08 to the sputum did not enhance the digestive action of the sodium hypochlorite nor add to its efficiency in enabling one to find acid-fast bacilli.—*Methods of concentrating acid-fast bacilli, H. J. Corper & C. R. Nelson, Am. J. Clin. Path., March, 1949, 19: 269.—(J. S. Woolley)*

Variant of Avian Bacillus.—An enlarged mesenteric node removed at autopsy contained an acid-fast organism with properties differing from those of human-type tubercle bacilli. The results of inoculation into guinea pigs, rabbits, and chickens, as well as the staining, morphological, and pathological characteristics, identified the organism as an attenuated form of the avian tubercle bacillus. This is the first instance of its kind in Brazil although similar findings have been reported both from Europe and the United States.—*Sôbre a presença de um bacilo tuberculoso tipo aviário atenuado, isolado a partir de um gânglio mesentérico proveniente da clínica humana, M. Fontes-Magarão, R. Fernandes, & J. Dauster, Clin. Tisiol., July–September, 1947, 2: 359.—(A. A. Moll)*

Serologic Reaction in Tuberculosis.—Earlier reports describe a serological reaction which has been manifested by all normal human beings and animals so far tested and which is referred to as a "universal serologic reaction." The technique employed in eliciting this reaction is based on precipitation with a lipid antigen and combines serum dilutions, NaCl concentrations, and incubation as variables. The test consists essentially of 5 quantitative Kahn tests at different salt concentrations. The results are read without incubation and after four hours' and twenty-four hours' incubation in the icebox. The reactions given by different normal persons are distinguishable within limits by individual serologic patterns and are believed to demonstrate the presence of natural antibodies to tissue lipids in human serum. Intensified precipitation is exhibited in syphilis, leprosy, and malaria. In one extensively studied case of tuberculosis, increased precipitation occurred in certain phases of the disease. During recovery the serological pattern approached normal. In 55 cases of far advanced tuberculosis there was no increased precipitation, indicating that the test is an antigen-antibody reaction; for it is well known that antibody production is a response requiring at least a minimal state of health. It is clear that the universal reaction cannot

be used as a diagnostic agent in the detection of tuberculosis although it may prove of value as an index of activity.—*Universal serological reaction with lipid antigen: VI. In tuberculosis, R. L. Kahn, Am. J. Clin. Path., May, 1949, 19: 419.*—(*J. S. Woolley*)

Public Health and Epidemiology

Tuberculosis in Childhood.—Tuberculous disease in children below the age of fifteen years is almost always the result of primary infection producing pathological effects by direct spread from the primary focus or glands or through the blood stream. If the fatal cases are divided into three-year age groups up to fifteen, the deaths for tuberculosis in each group comprise less than 10 per cent of the total deaths for that group. This is not an index of the risk of dying, once having acquired a primary infection. Between the ages of one and fifteen, tuberculosis is responsible for 15 per cent of all deaths. Below the age of three months, statistics are not a true index because there are certain causes of death inherent in the neonatal period. Taking the group three to twelve months of age, 3 per cent of deaths are due to tuberculosis. In the group one to five years, tuberculosis is the third leading cause of death; and in the group five to fifteen years, it is second only to violence. The above are statistics for all of England and Wales. New Castle-on-Tyne is an industrial city where the death rate for tuberculosis for all ages is 50 per cent greater than the national rate. In the group one to five years, tuberculosis is second only to acute respiratory disorders, while in the group five to fifteen years it is the leading cause of death. For the two groups combined it is the leading cause with 23.55 per cent of all deaths. In the one- to five-year group, representing 18,000 children, it was found that one-third of all deaths came from a group of 400 children living in a home where there was an open case. It is concluded that tuberculosis is an important cause of death in childhood.—*The mortality of primary tuberculosis in childhood, F. J. W. Miller, Lancet, August 13, 1949, 2: 275.*—(*A. G. Cohen*)

Minimal Tuberculosis in Adults.—Among 10,584 patients attending a tuberculosis dispensary at Rio di Janeiro, 431 (4.1 per cent) had minimal lesions. Routine examinations detected 505 other minimal cases, making 936 cases available for study. Direct examination of the sputum from 604 patients showed tubercle bacilli in only 5.9 per cent, as compared to 27.8 per cent in 792 examinations of gastric contents. Repeated examinations improved the results. Among the 936 patients, 819 (87.5 per cent) had exudative lesions and 117 productive lesions. The right lung was involved in 62.4 per cent of the cases, the left lung in 37.6 per cent, both lungs in 11.1 per cent. Infraclavicular lesions were found in 61.7 per cent, apical involvement in 25.7 per cent. Two-thirds of the exudative lesions were in the infraclavicular area and the other third in the apex. The reverse was the case with productive lesions. Among the 431 dispensary patients, 8.1 per cent were asymptomatic; 53.8 per cent of the 505 patients detected through routine surveys denied symptoms. The results of so-called home rest in 153 cases without collapse therapy were discouraging, 60.1 per cent of exudative cases and 26.7 per cent of productive cases becoming progressive. Surveys among contacts showed about four times as many initial cases as did routine mass surveys and about 40 per cent more minimal lesions.—*Sôbre o contrôle e a descoberta das lesões mínimas da tuberculose pulmonar do adulto, J. Rosemberg, Clín. Tisiol., July–September, 1948, 3: 279.*—(*A. A. Moll*)

BCG Vaccination in Brazil.—A study of 9,655 children receiving BCG vaccine and 6,312 nonvaccinated children living under identical conditions shows that BCG vaccination reduced the tuberculosis mortality to one-fifth that of the control group. The mortality in the treated group did not exceed 4.68 per cent in the first year of life. From 1927 to 1947, 222,054 infants were vaccinated at Rio de Janeiro. Now 23.54 per cent of newborn infants are vaccinated. A control study of families with 738 vaccinated and 694 unvaccinated children disclosed an approximate de-

crease of 50 per cent in tuberculosis morbidity and 80 per cent in tuberculosis mortality in the vaccinated group. In 61 infants exposed to home contagion, continued BCG vaccination was tried with the oral administration of 6 doses of 100 mg. of BCG vaccine at monthly intervals beginning as soon as possible after birth. At the end of a two-year period, not a single death from clinical tuberculosis had occurred and signs suggestive of tuberculosis developed in only 4. General mortality, all from nontuberculous causes, was 4.92 per cent.—*Prevenção da tuberculose in fantil no Rio de Janeiro por meio da vacinação oral com B. C. G., A. de Assis, Rev. brasil tuberc., May–June, 1948, 16: 145.*—(*A. A. Moll*)

Apparatus for BCG Vaccination.—An improved form of the multiple-puncture, spring-actuated apparatus for BCG vaccination permits easy detachment of the needle plate and end cap for heat sterilization. The improved outfit includes 4 needle plates and 4 end caps which are boilable. Thus, without interruption, mass BCG vaccination can proceed with completely sterile parts.—*A detachable spring-actuated multiple puncture apparatus for BCG vaccination, K. Birkhaug, Am. J. Clin. Path., May, 1949, 19: 492.*—(*J. S. Woolley*)

Correlationship of the Tuberculin Test with Other Factors.—Interpretation of either positive or negative results in tuberculin testing without due consideration of all other factors may lead to serious mistakes. Even in children, a positive reaction does not invariably imply active tuberculosis and on the other hand, a repeatedly negative result does not confirm by itself a diagnosis of tuberculosis. A relatively large number of children under five exposed to a tuberculous environment, mostly at home, were classified according to the results of skin tests and roentgenographic chest findings. Excluding 74 children because of inconclusive data, the group totaled 616. The largest fraction, with 328 children, had both a negative skin test and normal bronchopulmonary shadows. Only 116 (18.8 per cent) had both a positive tuberculin test and abnormal roentgenographic shadows. The second largest group of 125 children (20.3 per cent) revealed a positive tuberculin reaction and normal shadows and 47 (7.53 per cent) showed the reverse, i.e., a negative tuberculin reaction and abnormal roentgen shadows.—*Valoração do test tuberculínico e prognóstico da infecção tuberculosa na infância, G. de Matos, J. Feldman & A. de Oliveira-Lucena, Rev. brasil de tuberc., May–June, 1948, 16: 167.*—(*A. A. Moll*)

Skin Reaction to Tuberculin.—When a single tuberculin test is to be used for tuberculosis surveys, preference should be given to the skin reaction with 4 scarifications on the anterior surface of the arm. In 571 skin-positive adolescents, results of the skin reaction were compared with those given by the intradermal method. The intradermal reaction failed in 6 per cent with dilutions up to 1:1,000. This 6 per cent reacted positively to the 1:100 dilution. Among 219 skin-negative patients, 8 reacted positively to the 1:1,000 intradermal dilution and 71 to the 1:100 dilution. The scarification skin test was more sensitive at all dilutions under 1:100 but produced fewer severe reactions. When only 2 rather than 4 scarifications were used, about one-fourth of the positive cases failed to react.—*Sobre o valor da cutirreaç˜o e da intradermorreação em diversas diluiçoes na demonstraçao da alergia tuberculínica: Êstudo comparativo baseado em 790 adolescentes, A. de Carvalho, Clín. Tisiol., January–March, 1948, 3: 51.*—(*A. A. Moll*)

WHO and Tuberculosis.—The World Health Organization regards tuberculosis as a problem of great importance and has recommended several world-wide programs. Each country must determine the extent of its problem, its facilities, and its need for additional facilities. To aid recruitment and training, 34 scholarships have been made available and it is hoped to raise this to 50. The policy is to select senior officials in key positions in their Government's service and to give them a wider view of the work. Field service teams

use their own supplies and equipment initially and each country must agree to take over the project as soon as a sufficient number of its own personnel have been trained. In association with the International Children's Emergency Fund, 8,000,000 Europeans have been tuberculin tested and 3,000,000 vaccinated with BCG. The Organization desires to work in close harmony with the International Union against Tuberculosis by contacting national voluntary organizations through the accredited international agency.—*The World Health Organization and tuberculosis, J. B. McDougall, Brit. M. J., August 13, 1949, No. 4623: 379.*—(*R. W. Clarke*)

Unexplained Mortality Figures.—There has been an unexplained increase in the amount of pulmonary tuberculosis in Scotland since 1940. In 1947 the mortality rate rose to 0.66, the highest in twenty years. There was no parallel increase in England where the war damage to housing was even greater than in Scotland. It may be that the large Polish immigration into the latter region at the beginning of the war is responsible. This population may have harbored more tuberculosis and have a lower resistance to the disease. The nonrespiratory tuberculosis mortality rate shows a continuous decline.—*Tuberculosis: Certain unexplained mortality figures, W. Elliot, Brit. M. J., August 6, 1949, 4622: 297.*—(R. *W. Clarke*)

Tuberculosis in England and Wales.—Between 1918 and 1939 deaths from tuberculosis in England and Wales fell from 58,000 to 25,600 annually. In 1940 and 1941 they rose to 28,000; since 1941, they have fallen again, and in 1946 were 23,500. Admission of patients to tuberculosis institutions for treatment with reasonable promptness continues to present a serious problem, the cause being the persistent shortage of nurses and accommodations. On December 31, 1948, there were 35 units for mass miniature radiography. These units have examined 2,5000,000 persons, 94 per cent of whom were normal.—*Tuberculosis in England and Wales: Ministry statement, Brit. M. J., March 19, 1949, No. 4602: 493.*—(*R. W. Clarke*)

Tuberculosis Mortality in Urban Peru.—In 1947 the tuberculosis mortality per 100,000 in the eighteen largest Peruvian cities ranged from 875 to 236. Pulmonary tuberculosis caused over 80 per cent of the deaths in most communities. Tuberculosis accounted for more than 25 per cent of the general death rate in fourteen of the cities, falling below 20 per cent in only three cities. Males predominated in the mortality except in two cities. Tuberculosis has shown, on the whole a decreasing trend in the last five years, only four mountain cities exhibiting an increase since 1943.—*Estudios estadisticos sobre tuberculosis, Departamento de Tuberculosis, Ministerio de Salud Pública y Asistencia Social, Rev. brasil. de tuberc., July–December, 1948, 8: 249.*—(*A. A. Moll*)

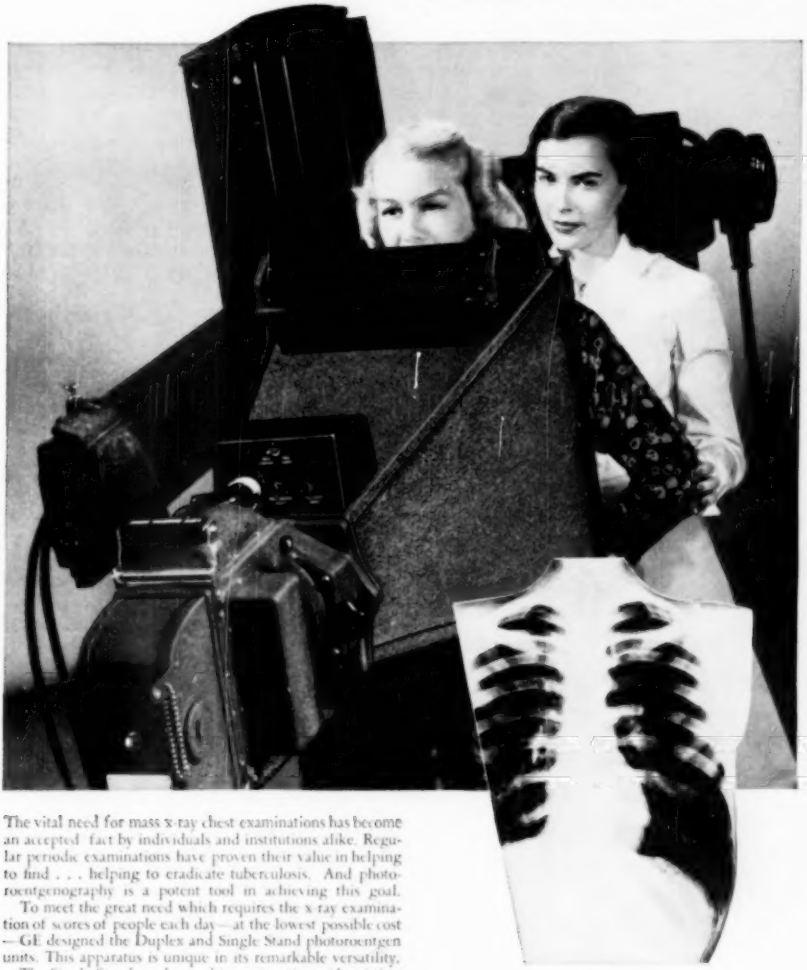
Netherlands Seal Sale.—This year the first Christmas seals will be issued by the Netherlands Antituberculosis Association. The seals will sell at ten cents each and the proceeds will be used to finance mass roentgenographic examinations.—*De Sluitzegel in de Strijd, Tegen de Tuberculose, Mej. G. C. Jos. Van Dienst, Tegen de Tuberculose, 1949, 35:*—(*J. van der Vate*)

Triethylene Glycol for Cold Prevention.—The germicidal effect of glycols had previously been reported. During the study period, triethylene glycol in a concentration of 0.0025 mg. per liter of air was introduced into an office during working hours. Bacterial counts showed no reduction in the number of airborne bacteria. Careful data showed no reduction in the absentee rate or in the incidence of upper respiratory infections among treated employees.—*An experiment with triethylene glycol vapor for the control of colds among office employees, W. J. McConnell, Industrial Med., May, 1949, 18: 192.*—(*A. G. Cohen*)

ERRATUM

In Vol. 60, No. 5, 1949, in the abstract "Avian Tuberculosis" the name of the author of the original article was given as "I. Gragsted" instead of "I. Dragsted."

An ideal answer for faster
T. B. CASE FINDING



The vital need for mass x-ray chest examinations has become an accepted fact by individuals and institutions alike. Regular periodic examinations have proven their value in helping to find . . . helping to eradicate tuberculosis. And photoroentgenography is a potent tool in achieving this goal.

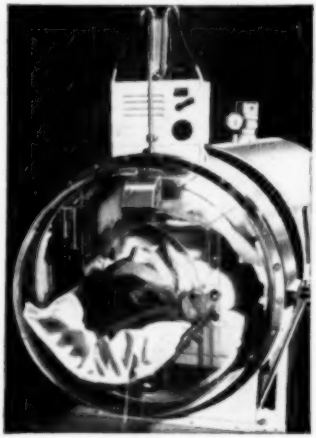
To meet the great need which requires the x-ray examination of scores of people each day—at the lowest possible cost—GE designed the Duplex and Single Stand photoroentgen units. This apparatus is unique in its remarkable versatility.

The Single Stand can be used in conjunction with existing diagnostic facilities, while the Duplex model is a complete x-ray unit in itself . . . making possible the selection of a particular combination to meet the specific need!

For illustrated information about these photoroentgen units, see your local GE representative, or write General Electric X-Ray Corporation, Dept. **S-2,** Milwaukee 14, Wis.

GENERAL ELECTRIC
X-RAY CORPORATION

MORE THAN HALF
of those treated in the
EMERSON LUNG IMMOBILIZER



By Barach's method of total lung rest have shown successful results—"disappearance of cavity with negative sputum"... "arrest of their disease with ability to work" etc.—according to published reports. And these were cases of advanced, bilateral tuberculosis which had previously proved resistant to usual measures. Immobilization therapy is not expensive, is generally not long, and does not preclude other procedures.

This "intensification of the principle of bed rest" is an important new resource for the physician.

For information and reprints, write:

J. H. EMERSON CO.

Makers of the Naclerio Support for Prone-Position Surgery

22 Cottage Park Ave., Cambridge 40, Mass.

OAK RIDGE SANATORIUM

GREEN SPRINGS, OHIO

for
TUBERCULOSIS

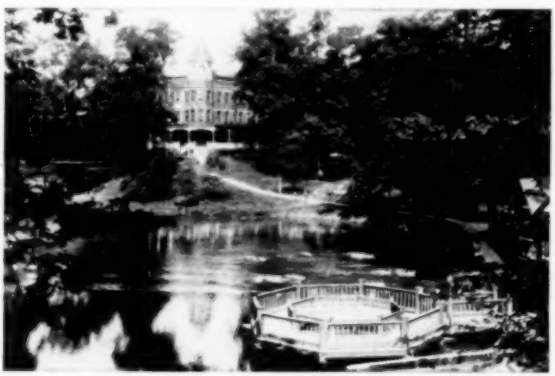
•

Diagnosis
Treatment

•

SITUATED in the beautiful springs country of Northern Ohio, this modern Sanatorium offers not only up-to-date treatment for all forms of Tuberculosis but a setting of utmost beauty and restfulness for the convalescent. General Hospital Facilities with complete Surgery . . . Modern Steam-heated Rooms . . . Graduate Nurses . . . Personal Care for Every Patient.

Natural Mineral Spring
(8,000,000 gallons per day.)
Artesian Well



Reasonable rates

PAUL M. HOLMES, M.D., Medical Director
JOHN J. GEDERT, M.D., Resident Physician
GEO. S. BOWERS, M.D., Internist
ELEANOR BLIVEN, R.N., Supt. Nurses
ALEX C. JOHNSON, Pres. & Gen. Mgr.
M. M. RIDDLE, M.D., Eye, Ear, Nose and Throat
WM. NEILL, M.D., Thoracic Surgeon
OTTO MUHME, M.D., Thoracic Surgeon

Thirty-sixth Session:

TRUDEAU SCHOOL OF TUBERCULOSIS

AT SARANAC LAKE, N. Y.

In the Spring of 1950

Four Weeks Course Beginning

MAY 1

Schedule of Instruction includes the history, etiology and epidemiology; pathology, bacteriology, physiology and biochemistry; clinics and bedside teaching, including surgical and special treatments; fungus infections; pneumoconioses; pulmonary tumors, and differential diagnosis of other lung disorders.

Clinical Facilities Available: Trudeau Sanatorium, Ray Brook State Hospital, Stony Wold Sanatorium, Will Rogers Memorial, Sanatorium Gabriels, V. A. Hospital at Sunmount, N. Y., and the General Hospital of Saranac Lake (chiefly surgical).

Research Facilities Available: Saranac Laboratory; Trudeau Laboratory; Physiology and Biochemistry Laboratories; Ray Brook State Hospital Laboratory, and the X-Ray Laboratories of Trudeau, Ray Brook and Stony Wold Sanatoria.

Lectures, Demonstrations and Conferences conducted by the Staffs and other experienced local clinicians. By invitation, recognized authorities give lectures on specific subjects.

Students Participate Actively in the study and discussion of cases.

Fee for Four Weeks Course is $100.00. The Trudeau School has been approved for training under Public Laws 16 and 346, for which registration should be made in advance. The class is limited, therefore early application is advised. A few scholarships are available.

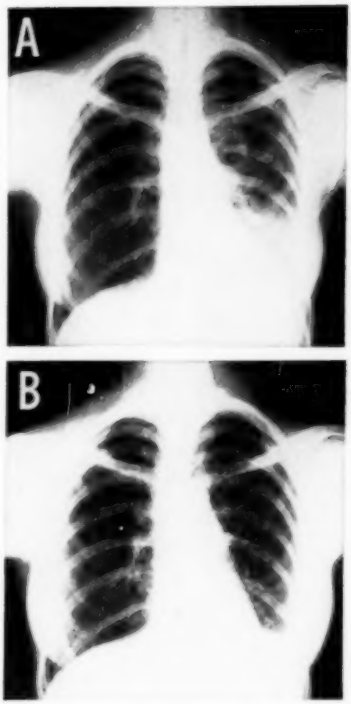
For Schedule and other information write to
Secretary, TRUDEAU SCHOOL, Saranac Lake, N. Y.

Effective Chemotherapy in TUBERCULOSIS

Streptomycin or Dihydrostreptomycin, used alone or in combination with para-aminosalicylic acid, is recognized as a valuable and, in some instances, an essential adjuvant in the treatment of selected types and stages of tuberculosis.

Para-aminosalicylic acid is capable of inhibiting or significantly delaying the emergence of bacterial resistance to streptomycin or dihydrostreptomycin.

These drugs are not to be regarded as substitutes for traditional therapeutic methods. Rather, they serve best when properly integrated with bed rest and, where necessary, collapse measures or other forms of surgery.



A. Before Treatment
(9 days prior to Dihydrostreptomycin therapy) Diffuse lobular tuberculous pneumonia, lower half of left lung; thin-walled cavity above hilus (3 x 3.5 cm.).

B. After 3 Mos. Treatment
(2 days after discontinuance of Dihydrostreptomycin) Considerable clearing of acute exudative process in the diseased lung; cavity smaller and wall thinner.

Detailed literature on the subject of chemotherapy in tuberculosis will be supplied upon request.

MERCK & CO., Inc.
Manufacturing Chemists
RAHWAY, N. J.

Merck Antitubercular Agents

Streptomycin Calcium Chloride Complex Merck

Para-Aminosalicylic Acid Merck (PAS)

Dihydrostreptomycin Sulfate Merck

ALUM ROCK SANATORIUM

SAN JOSE, CALIFORNIA

Telephone: CLayburn 8-4921

A non-profit sanatorium for the treatment of Tuberculosis and other diseases of the chest.

Medical Director
Buford H. Wendrip, M.D.
Associate Medical Director
C. G. Scarborough, M.D.

Visiting Medical Staff:

Harold Gayon Trimble, M.D., Oakland
Cabot Brown, M.D., San Francisco
J. Lloyd Eaton, M.D., Oakland
Ira Gowley, M.D., Oakland
Glenroy N. Pierce, M.D., San Francisco
Gerald L. Crenshaw, M.D., Oakland
James Robert Wood, M.D., Oakland

A SPECIAL ANNOUNCEMENT TO SUBSCRIBERS

For more than five years, the American Review of Tuberculosis has been waging a losing battle against advancing production costs. The prices of paper, type-setting, presswork, and binding have steadily mounted, and the end is not yet in sight.

With reluctance, therefore, we are forced to announce an increase in the subscription rate from $8.00 to $10.00, *effective January 1950.*

THE AMERICAN REVIEW OF TUBERCULOSIS
1790 Broadway, • New York, N. Y.

Maryknoll
SANATORIUM

(Maryknoll Sisters)

MONROVIA, CALIFORNIA

• A Sanatorium for the treatment of tuberculosis and other diseases of the lungs. Located in the foothills of the Sierra Madre Mountains, Southern exposure. Accommodations are private, modern, and comfortable. Rates are moderate. General care of patient is conducive to physical and mental well being.

SISTER MARY PIETA
Superintendent

E. W. HAYES, M.D.
Medical Director

"PULMONARY TUBERCULOSIS"

Dr. Hans Jacob Ustvedt brilliantly discusses modern ideas concerning the psychology and treatment of tuberculosis. His close work with Scheel and Heimbock of Oslo, their epoch-making work and notable advances in tuberculosis studies make this book valuable for General Practitioners, students and tuberculosis specialists. Generously illustrated, yours for only $6.50.

"BOVINE TUBERCULOSIS"

By John Francis, B.Sc., M.R.C.V.S., here's a priceless hand book for the Veterinary Surgeon, medical worker interested in the study of safe dairy products, for the researcher comparing tuberculosis pathology. These 220 pages of comprehensive data with convenient tables, references and illustrations cover the history and distribution of the disease and various methods of vaccination. Yours for only $4.50.

"TUBERCLE"

A convenient way to keep abreast of tuberculosis topics, here is the official journal of the British Tuberculosis Association. Published for 30 years, this notable monthly is regarded as one of the leading periodicals devoted exclusively to every aspect of tuberculosis and ancillary diseases. Yearly subscription only $6.00.

Order one or all three NOW! (Money back guarantee)

Dept. AR.T. 1

Staples Press, Inc.

70 East 45th Street, New York 17, N. Y.

"Vitamins In Medical Practice"

Complete Comprehensive Study of Vitamins By J. Shafer, M.D., M.R.C.P. Devoted to practical issues of vitamin therapy in clinical practice. Only $4.50.

THE PANRAY CORP. announces

A Potentially Significant Advance in PAS therapy

PARASAL SODIUM TABLETS, 0.69 Gm.

Each tablet contains 0.69 gm. sodium para aminosalicylate dehydrate—equivalent to 0.5 gm. active para aminosalicylic acid *with the following desirable characteristics:*

- **EXTREME PURITY and UNIFORMITY**
- **NEUTRAL pH**
- **RAPID DISINTEGRATION and ABSORPTION**
- **LOWER TOXICITY**
- **GREATER PAS ACTIVITY**

It is hoped that these Parasal Sodium tablets, made from a very special grade of PAS and the result of a year's intensive research, will *minimize* or *completely eliminate gastro-intestinal side effects*, thus obviating the need for enteric coatings or alkaline additives.

Parasal Sodium tablets (and powder) are available to qualified investigators for research and evaluation. Write today for literature.

PARASAL is Panray's brand of PAS (Para Aminosalicylic Acid) as manufactured by Hexagon Laboratories, Inc., and distributed exclusively to the medical profession by The Panray Corp.

THE Panray CORP • 340 CANAL STREET, NEW YORK 13, N. Y.

CUSTOM MANUFACTURERS AND DISTRIBUTORS OF FINE ORGANIC CHEMICALS